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(54) Title: CELLS CAPABLE OF DIFFERENTIATING INTO HEART MUSCLE CELLS

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(57) Abstract: Methods of isolating, purifying, culturing and differentiation-inducing cells which are capable of differentiating into heart muscle cells; a method of proliferating cells which are capable of differentiating into heart muscle cells and a method of regulating the differentiation thereof into heart muscle cells by using various cytokines, transcriptional factors, etc.; a method of acquiring a surface antigen specific to cells which are capable of differentiating into heart muscle cells; a method of acquiring a gene encoding this surface antigen; a method of acquiring an antibody specific to the above surface antigen; a method of acquiring a protein and a gene participating in the proliferation of cells which are capable of differentiating into heart muscle cells and differentiation thereof into heart muscle cells; remedies for various heart diseases with the use of cells which are capable of differentiating into heart muscle cells; and a method of inducing the differentiation of various cells (nerve cells, liver cells, fat cells, skeletal muscle cells, vascular endothelial cells, osteoblasts, etc.) and tissues by using cells which are capable of differentiating into heart muscle cells.

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ABSTRACT

The present invention relates to methods for isolation, purification, expansion, and differentiation of cells having the potential to differentiate into cardiomyocytes. Furthermore, the present invention relates to methods for proliferating cells having the potential to differentiate into cardiomyocytes and for regulating their differentiation into cardiomyocytes using various cytokines and transcription factors. Moreover, the present invention relates to a method for obtaining surface antigens specific for cells having the potential to differentiate into cardiomyocytes, a method for obtaining genes encoding the surface antigens, a method for obtaining antibodies specific for the surface antigens, and a method for obtaining a protein and a gene controlling the proliferation of cells having the potential to differentiate into cardiomyocytes and their differentiation into cardiomyocytes. Also, the present invention relates to therapeutic agents for various heart diseases containing cells having the potential to differentiate into cardiomyocytes. Still furthermore, the present invention relates to a method for differentiating various cells and tissues such as neural cells, hepatocytes, adipocytes, skeletal muscle cells, vascular endothelial

cells and osteoblasts, using cells having the potential to differentiate into cardiomyocytes.

SPECIFICATION

THE CELL HAVING THE POTENTIALITY OF DIFFERENTIATION INTO CARDIOMYOCYTES

TECHNICAL FIELD

The present invention relates to methods for isolation, purification, expansion, and differentiation of cells having the potential to differentiate into cardiomyocytes. Furthermore, the present invention relates to methods for proliferating cells having the potential to differentiate into cardiomyocytes and for regulating their differentiation into cardiomyocytes using various cytokines and transcription factors. Moreover, the present invention relates to a method for obtaining surface antigens specific for cells having the potential to differentiate into cardiomyocytes, a method for obtaining genes encoding the surface antigens, a method for obtaining antibodies specific for the surface antigens, and a method for obtaining a protein and a gene controlling the proliferation of cells having the potential to differentiate into cardiomyocytes and their differentiation into cardiomyocytes. Also, the present invention relates to therapeutic agents for various heart diseases containing cells having the potential to differentiate into cardiomyocytes.

BACKGROUND ART

Cardiomyocytes actively divide into daughter cells with spontaneous beating before birth. However, they lose the proliferative activity after birth and never acquire the division potentiality again unlike hepatocytes. Furthermore, unlike skeletal muscles, they does not have undifferentiated precursor cells such as satellite cells. Therefore, when cardiomyocytes are necrotized by myocardial infarction, myocarditis, senility etc., hypertrophy of the remaining cardiomyocytes occurs *in vivo* instead of cell division. Cardiac hypertrophy is a physiological adaptation at the initial stage, but when coupled with the fibrosis of stroma by the growth of cardiac fibroblasts, it comes to lower the diastolic function and the systolic function of heart itself, leading to heart failure. Therapy so far developed for heart failure caused by myocardial infarction, etc. is mainly symptomatic therapy, for example, intensification of the cardiac systolic function, alleviation of the pressure overload and the volume load on heart using a vasodilator drug, and decrease of blood flow using of a diuretic. On the other hand, heart transplantation is alternative therapy for severe heart failure, but is not generally adopted as a common treatment because of problems such as shortage of heart donors, difficulty in judging cerebral death, immune

rejection and a great rise in medical cost. At present, heart diseases are the third cause of mortality in Japan (*Annual Report on Health and Welfare*, 1998), and thus success in regeneration of lost cardiomyocytes will lead to a great advance in medical welfare.

As a cell line retaining the characteristics of cardiomyocytes, AT-1 cell line has been obtained from the atrial tumor of the transgenic mouse expressing SV40 promoter large T antigen under the control of atrial natriuretic hormone promoter (*Science*, 239: 1029-1038 (1988)). However, this cell line forms tumors when transplanted *in vivo* and thus is inappropriate for cell transplantation. Under these circumstances, the following methods have been proposed for reconstructing myocardium.

The first method is conversion of cells other than cardiomyocytes into cardiomyocytes, which has been proposed on the analogy of the conversion of fibroblasts into skeletal muscle cells by the introduction of MyoD. Although a successful result has been reported with P19 cell which is a murine embryonal carcinoma cell (*Cell Struc. & Func.*, 21: 101-110 (1996)), there has been no report on success with non-carcinomatous cells.

The second method is restoration of proliferative activity to cardiomyocytes, which is based on the fact that beating cardiomyocytes can proliferate in the fetus. No successful example of this method has been reported yet.

The third method is induction of cardiomyocytes from undifferentiated stem cells. It has already been demonstrated that cardiomyocytes can be differentiated from embryonic stem cells (ES cells), but there still remain the problems of carcinoma formation and immune rejection by embryonic stem cells transplanted into an adult tissue. (*Nature Biotechnology*, 17: 139-142 (1999)).

In order to practically utilize embryonic stem cells in medical treatments, it is essential to develop a technique for highly purifying at least cardiomyocyte precursor cells or cardiomyocytes. As for the problem of immune rejection, the possibility of solving the problem by the cloning technique has been suggested, but it is difficult to apply this technique to general medical treatments because of its complicated operation.

It has also been proposed to transplant undifferentiated cardiomyocyte precursor cells obtained from an aborted fetus, and it is known that such cells effectively function as cardiomyocytes in an experiment using animals (*Science*, 264: 98-101 (1994)). However, it is difficult to obtain a large amount of cardiomyocyte precursor cells in this method, and the method is hardly applicable to general medical treatments also from an ethical viewpoint.

It is known that there exist mesenchymal stem cells besides hematopoietic stem cells and vascular stem cells in

adult bone marrow and that mesenchymal stem cells can be induced to differentiate into osteocytes, chondrocytes, tendon cells, ligament cells, skeletal muscle cells, adipocytes, stromal cells and hepatic oval cells (*Science*, 284: 143-147 (1999); *Science*, 284: 1168-1170 (1999)). On the other hand, it has been recently reported that the cells obtained from the bone marrow of an adult mouse can be induced to differentiate into cardiomyocytes (*J. Clinical Investigation*, 103: 10-18 (1999)). This report suggests that the cell therapy which comprises transplanting cells which are obtained from bone marrow fluid taken from a patient followed by in vitro expansion and drug treatment to the damaged part of the patient's heart can be a practical medical treatment (*J. Clinical Investigation*, 103: 591-592 (1999)). However, this report merely indicates that a part of the immortalized cells established from the bone marrow of an adult mouse can differentiate into cardiomyocytes. Furthermore, the report fails to isolate, selectively proliferate, and efficiently differentiate the adult bone marrow cells having the potential to differentiate into cardiomyocytes (*J. Clinical Investigation*, 103: 591-592 (1999)).

Antibodies which recognize various surface antigens are used to isolate the target cells from the tissue of vital body. For example, it is known that immature hematopoietic stem cells have the characteristics of

CD34+/CD38-/HLA-DR-/CD90 (Thy-1)+, and CD38 is expressed while CD90(Thy-1) disappears in the process of differentiation (*Protein, Nucleic Acid, Enzyme*, 45: 13, 2056-2062 (2000)). In vascular endothelial cells, markers such as CD34, CD31, Flk-1, Tie-2, E-selectin, etc. are expressed (*Molecular Cardiovascular Disease*, 1(3): 294-302 (2000)). In bone marrow mesenchymal stem cells, markers such as CD90, CD105, CD140, etc. are expressed (*Science*, 284: 143-147 (1999); *Science*, 284: 1168-1170 (1999)). However, no surface marker of stem cells capable of inducing both myocardium and vascular endothelial cells is known.

DISCLOSURE OF THE INVENTION

Under the circumstances, a need exists for the development of therapy for heart diseases which therapy is safer and more established than those currently available. It is useful to select cells having the potential to differentiate into cardiomyocytes from a vital tissue such as bone marrow cells or the like or umbilical blood and to control the growth or differentiation of the cells for the development of myocardium-regenerating therapy using vital cells such as bone marrow-derived cells or the like or umbilical blood. For this purpose, it is necessary to separate the cells having the potential to differentiate into cardiomyocytes and to identify cytokines or

transcription factors participating in the growth or differentiation of such cells.

The present inventors have made intensive studies aiming at solving the above problems and have obtained the following results. Specifically, various cell lines were obtained by separating mouse bone marrow-derived cells to single cell level. Then, various cell lines have characterized by their potential to differentiate into cardiomyocytes by treating each cell line with 5-azacytidine. Next, by labeling the thus obtained cell line using a retrovirus vector which expresses a GFP (green fluorescent protein) and tracing the cells using a fluorescence microscope, it has been found that the bone marrow-derived cells are pluripotent stem cells which can differentiate into at least two different cells, i.e., cardiomyocytes and adipocytes. Furthermore, it has been found that the stem cells can be differentiated into cardiomyocytes, adipocytes and skeletal muscle cells stochastically by addition of not only 5-azacytidine but also other genomic DNA-demethylating agents, such as DMSO (dimethyl sulfoxide), indicating that demethylation of genomic DNA is effective in inducing the differentiation of bone marrow-derived cells into cardiomyocytes. Moreover, it was found that the expression of myocardium-specific genes, ANP (atrial natriuretic peptide) and cTnI (cardiac Troponin I), can be expressed in the bone marrow-derived

cells by adding at least one cytokine of four cytokines, FGF-8, ET1, midkine and BMP4, combined with 5-azacytidine. Also, it was found that differentiation of the bone marrow-derived cells into cardiomyocytes can be promoted about 50-fold by the forced expression of two transcriptional factors, Nkx2.5 and GATA4, in these bone marrow-derived cells using virus vectors followed by 5-azacytidine treatment. Furthermore, it was found that the expression of ANP and cTnI, which are myocardium-specific genes, in the bone marrow-derived cells can be specifically promoted by culturing these bone marrow-derived cells in a culture dish coated with a cardiomyocyte-derived extracellular substrate. Moreover, it was found that the formation of myocardium from the bone marrow-derived cells can be about 10 times or more promoted by co-culturing the bone marrow-derived cells together with primarily cultured cells derived from myocardium. Moreover, it was found that differentiation of the bone marrow-derived cells into cardiomyocytes can be promoted about 500-fold when the forced expression of two transcription factors Nkx2.5 and GATA4 in the bone marrow-derived cells using virus vectors and co-culturing these cells with cardiomyocytes were combined.

Subsequently, the differentiation potency of the bone marrow-derived cells was examined by a transplantation experiment. First, the bone marrow-derived cells were

transplanted into an adult mouse heart and it was thus found that these bone marrow-derived cells were differentiated into myocardia and vessels. Furthermore, the bone marrow-derived cells were transplanted into an adult mouse muscle and it was thus found that these bone marrow-derived cells could form skeletal muscles. When the bone marrow-derived cells were transplanted into a mouse blastocyst, tissues derived from these transplanted cells were formed in the central nervous system, liver and heart of the mouse. The central nervous system, liver and heart are tissues of the ectoderm, endoderm and mesoderm, respectively.

These results indicate that the bone marrow-derived cells found in the present invention have properties different from those possessed by hematopoietic stem cells which are differentiated into only hematopoietic stem tissue present in bone marrow and from those possessed by mesenchymal stem cell which are differentiated into only dorsal mesoderm tissue such as skeletal muscle, adipocytes, bone and the like known in the art, that is, a totipotency of differentiating into all of the three germ layers including the ectoderm, mesoderm and endoderm.

Furthermore, the inventors analyzed the expression of surface antigens of bone marrow-derived cells using antibodies which recognize hematopoietic cell surface antigens, CD34, CD117, CD14, CD45, CD90, Sca-1, Ly6c and

Ly6g, antibodies which recognize vascular endothelial cell surface antigens, Flk-1, CD31, CD105 and CD144, antibodies which recognize a mesenchymal cell surface antigen, CD140, antibodies which recognize integrin surface antigens, CD49b, CD49d, CD29 and CD41, and antibodies which recognize matrix receptors, CD54, CD102, CD106 and CD44, and the like in these bone marrow cells of the present invention and thus found that they are totipotent stem cells exhibiting a quite novel expression form having been unknown, thereby completing the present invention.

Specifically, the present invention provides the following (1)-(91):

- (1) A cell which has been isolated from a living tissue or umbilical blood, and which has the potential to differentiate into at least a cardiomyocyte.
- (2) The cell according to (1), wherein the living tissue is bone marrow.
- (3) The cell according to (1) or (2), wherein the cell is a multipotent stem cell.
- (4) The cell according to any one of (1) to (3), wherein the cell is a multipotent stem cell which differentiates into at least a cardiomyocyte and a vascular endothelial cell.
- (5) The cell according to any one of (1) to (4), wherein the cell is a multipotent stem cell which

differentiates into at least a cardiomyocyte, an adipocyte, a skeletal muscle cell, an osteoblast, and a vascular endothelial cell.

(6) The cell according to any one of (1) to (5), wherein the cell is a multipotential stem cell which differentiates into at least a cardiomyocyte, an adipocyte, a skeletal muscle cell, an osteoblast, a vascular endothelial cell, a nervous cell, and a hepatic cell.

(7) The cell according to any one of (1) to (3), wherein the cell is a multipotential stem cell which differentiates into any cell in adult tissues.

(8) The cell according to any one of (1) to (7), wherein the cell is CD117-positive and CD140-positive.

(9) The cell according to (8), wherein the cell is further CD34-positive.

(10) The cell according to (9), wherein the cell is further CD144-positive.

(11) The cell according to (9), wherein the cell is further CD140-negative.

(12) The cell according to (8), wherein the cell is CD34-negative.

(13) The cell according to (12), wherein the cell is further CD144-positive.

(14) The cell according to (12), wherein the cell is further CD144-negative.

(15) The cell according to (10), wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

(16) The cell according to (11), wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

(17) The cell according to (12), wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

(18) The cell according to (13), wherein the cell is further CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative, and CD44-positive.

(19) The cell according to (1), which does not take up Hoechst 33342.

(20) A cardiomyocyte precursor which differentiates into only cardiomyocyte induced from the cell according to any one of (1) to (19).

- (21) The cell according to any one of (1) to (20), which has the potential to differentiate into a ventricular cardiac muscle cell.
- (22) The cell according to any one of (1) to (20), which has the potential to differentiate into a sinus node cell.
- (23) The cell according to any one of (1) to (20), wherein the vital tissue or umbilical blood is derived from a mammal.
- (24) The cell according to (23), wherein the mammal is selected from the group consisting of a mouse, a rat, a guinea pig, a hamster, a rabbit, a cat, a dog, a sheep, a swine, cattle, a goat and a human.
- (25) The cell according to any one of (1) to (8), which is mouse bone marrow-derived multipotential stem cell BMSC (FERM BP-7043).
- (26) The cell according to any one of (1) to (25), which has the potential to differentiate into a cardiomyocyte by demethylation of a chromosomal DNA of the cell.
- (27) The cell according to (26), wherein the demethylation is carried out by at least one selected from the group consisting of demethylase, 5-azacytidine, and dimethyl sulfoxide, DMSO.
- (28) The cell according to (27), wherein the demethylase comprises the amino acid sequence represented by SEQ ID NO:1.

(29) The cell according to any one of (1) to (28), wherein the differentiation is accelerated by a factor which is expressed in a cardiogenesis region of a fetus or a factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus.

(30) The cell according to (29), wherein the factor which is expressed in a cardiogenesis region of a fetus or the factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus is at least one selected from the group consisting of a cytokine, an adhesion molecule, a vitamin, a transcription factor, and an extracellular matrix.

(31) The cell according to (30), wherein the cytokine is at least one selected from the group consisting of a platelet-derived growth factor, PDGF; a fibroblast growth factor-8, FGF-8; an endothelin 1, ET1; a midkine; and a bone morphogenetic factor, BMP-4.

(32) The cell according to (31), wherein the PDGF, FGF-8, ET1, midkine, and BMP-4 comprise the amino acid sequence represented by SEQ ID NO:3 or 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively.

(33) The cell according to (30), wherein the adhesion molecule is at least one selected from the group consisting of a gelatin, a laminin, a collagen, and a fibronectin.

(34) The cell according to (30), wherein the vitamin is retinoic acid.

(35) The cell according to (30), wherein the transcription factor is at least one selected from the group consisting of Nkx2)5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1.

(36) The cell according to (35), wherein the Nkx2)5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1 comprise the amino acid sequence represented by SEQ ID NO:9, the amino acid sequence represented by SEQ ID NO:11, the amino acid sequence represented by SEQ ID NO:13, the amino acid sequence represented by SEQ ID NO:15, the amino acid sequence represented by SEQ ID NO:17, the amino acid sequence represented by SEQ ID NO:19, the amino acid sequence represented by SEQ ID NO:21, the amino acid sequence represented by SEQ ID NO:23, the amino acid sequence represented by SEQ ID NO:25, the amino acid sequence represented by SEQ ID NO:27, the amino acid sequence represented by SEQ ID NO:29, and the amino acid sequence represented by SEQ ID NO:62, respectively.

(37) The cell according to (30), wherein the extracellular matrix is an extracellular matrix derived from a cardiomyocyte.

(38) The cell according to any one of (1) to (28), wherein the differentiation is inhibited by a fibroblast growth factor-2, FGF-2.

(39) The cell according to (38), wherein the FGF-2 comprises the amino acid sequence represented by SEQ ID NO:7 or 8.

(40) The cell according to any one of (1) to (28), which is capable of differentiating into a cardiomyocyte or a blood vessel by transplantation into a heart.

(41) The cell according to any one of (1) to (28), which is capable of differentiating into a cardiac muscle by transplantation into a blastocyst or by co-culturing with a cardiomyocyte.

(42) The cell according to any one of (1) to (28), which is capable of differentiating into an adipocyte by an activator of a nuclear receptor, PPAR- γ .

(43) The cell according to (42), wherein the activator is a compound having a thiazolidione skeleton.

(44) The cell according to (43), wherein the compound is at least one selected from the group consisting of troglitazone, pioglitazone, and rosiglitazone.

(45) The cell according to any one of (1) to (28), which is capable of differentiating into a nervous cell by

transplantation into a blastocyst or by transplantation into an encephalon or a spinal cord.

(46) The cell according to any one of (1) to (28), which is capable of differentiating into a hepatic cell by transplantation into a blastocyst or by transplantation into a liver.

(47) A method for differentiating the cell according to any one of (1) to (28) into a cardiac muscle, comprising using a chromosomal DNA-dimethylating agent.

(48) A method for redifferentiating the cell according to (9) into the cell according to (12), comprising using a chromosomal DNA-dimethylating agent.

(49) A method for redifferentiating a cell which is CD117-negative and CD140-positive into the cell according to (8), comprising using a chromosomal DNA-dimethylating agent.

(50) The method according to (48) or (49), wherein the chromosomal DNA-dimethylating agent is selected from the group consisting of a demethylase, 5-azacytidine, and DMSO.

(51) The method according to (50), wherein the demethylase comprises the amino acid sequence represented by SEQ ID NO:1.

(52) A method for differentiating the cell according to any one of (1) to (28) into a cardiac muscle, comprising using a factor which is expressed in a cardiogenesis region

of a fetus or a factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus.

(53) The method according to (52), wherein the factor which is expressed in a cardiogenesis region of a fetus or the factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus is at least one selected from the group consisting of a cytokine, an adhesion molecule, a vitamin, a transcription factor, and an extracellular matrix.

(54) The method according to (53), wherein the cytokine is at least one selected from the group consisting of a platelet-derived growth factor, PDGF; a fibroblast growth factor-8, FGF-8; an endothelin 1, ET1; a midkine; and a bone morphogenetic factor, BMP-4.

(55) The method according to (54), wherein the PDGF, FGF-8, ET1, midkine, and BMP-4 comprise the amino acid sequence represented by SEQ ID NO:3 or 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively.

(56) The method according to (53), wherein the adhesion molecule is at least one selected from the group consisting of a gelatin, a laminin, a collagen, and a fibronectin.

(57) The method according to (53), wherein the vitamin is retinoic acid.

(58) The method according to (53), wherein the transcription factor is at least one selected from the group consisting of Nkx2)5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1.

(59) The method according to (58), wherein the Nkx2)5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1 comprise the amino acid sequence represented by SEQ ID NO:9, the amino acid sequence represented by SEQ ID NO:11, the amino acid sequence represented by SEQ ID NO:13, the amino acid sequence represented by SEQ ID NO:15, the amino acid sequence represented by SEQ ID NO:17, the amino acid sequence represented by SEQ ID NO:19, the amino acid sequence represented by SEQ ID NO:21, the amino acid sequence represented by SEQ ID NO:23, the amino acid sequence represented by SEQ ID NO:25, the amino acid sequence represented by SEQ ID NO:27, the amino acid sequence represented by SEQ ID NO:29, the amino acid sequence represented by SEQ ID NO:62, respectively.

(60) The method according to (53), wherein the extracellular matrix is an extracellular matrix derived from a cardiomyocyte.

(61) A method for differentiating the cell according to any one of (1) to (28) into an adipocyte, comprising using an activator of a nuclear receptor, PPAR- γ .

(62) The method according to (61), wherein the activator is a compound having a thiazolidione skeleton.

(63) The method according to (62), wherein the compound is at least one selected from the group consisting of troglitazone, pioglitazone, and rosiglitazone.

(64) A myocardium-forming agent, comprising, as an active ingredient, a chromosomal DNA-demethylating agent.

(65) The myocardium-forming agent according to (64), wherein the chromosomal DNA-demethylating agent is at least one selected from the group consisting of a demethylase, 5-azacytidine, and DMSO.

(66) The myocardium-forming agent according to (65), wherein the demethylase comprises the amino acid sequence represented by SEQ ID NO:1.

(67) A myocardium-forming agent, comprising, as an active ingredient, a factor which is expressed in a cardiogenesis region of a fetus or a factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus.

(68) The myocardium-forming agent according to (67), wherein the factor which is expressed in a cardiogenesis region of a fetus or the factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus is at least one selected from the group consisting of a cytokine, an adhesion molecule, a vitamin, a transcription factor, and an extracellular matrix.

(69) The myocardium-forming agent according to (68), wherein the cytokine is at least one selected from the group consisting of a platelet-derived growth factor, PDGF; a fibroblast growth factor-8, FGF-8; an endothelin 1, ET1; a midkine; and a bone morphogenetic factor, BMP-4.

(70) The myocardium-forming agent according to (69), wherein the PDGF, FGF-8, ET1, midkine, and BMP-4 comprise the amino acid sequence represented by SEQ ID NO:3 or 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively.

(71) The myocardium-forming agent according to (68), wherein the adhesion molecule is selected from the group consisting of a gelatin, a laminin, a collagen, and a fibronectin.

(72) The myocardium-forming agent according to (71), wherein the vitamin is retinoic acid.

(73) The myocardium-forming agent according to (68), wherein the transcription factor is at least one selected from the group consisting of Nkx2)5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1.

(74) The myocardium-forming agent according to (73), wherein the Nkx2)5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1 comprise

the amino acid sequence represented by SEQ ID NO:9, the amino acid sequence represented by SEQ ID NO:11, the amino acid sequence represented by SEQ ID NO:13, the amino acid sequence represented by SEQ ID NO:15, the amino acid sequence represented by SEQ ID NO:17, the amino acid sequence represented by SEQ ID NO:19, the amino acid sequence represented by SEQ ID NO:21, the amino acid sequence represented by SEQ ID NO:23, the amino acid sequence represented by SEQ ID NO:25, the amino acid sequence represented by SEQ ID NO:27, the amino acid sequence represented by SEQ ID NO:29, and the amino acid sequence represented by SEQ ID NO:62, respectively.

(75) The myocardium-forming agent according to (68), wherein the extracellular matrix is an extracellular matrix derived from a cardiomyocyte.

(76) A method for regenerating a heart damaged by a heart disease, comprising using the cell according to any one of (1) to (46).

(77) An agent for cardiac regeneration, comprising, as an active ingredient, the cell according to any one of (1) to (46).

(78) A method for specifically transfecting a wild-type gene corresponding to a mutant gene in a congenital genetic disease to a myocardium, comprising using the cell according to any one of (1) to (46) into which the wild-

type gene corresponding to a mutant gene in a congenital genetic disease of a heart has been introduced.

(79) A therapeutic agent for a heart disease, comprising, as an active ingredient, the cell according to any one of (1) to (46) into which a wild-type gene corresponding to a mutant gene in a congenital genetic disease of a heart has been introduced.

(80) A method for producing an antibody which specifically recognizes the cell according to any one of (1) to (46), comprising using the cell as an antigen.

(81) A method for isolating a cell having the potential to differentiate into a cardiomyocyte according to any one of (1) to (46), comprising using an antibody obtained by the method according to (80).

(82) A method for obtaining a surface antigen specific for the cell according to any one of (1) to (46), comprising using the cell.

(83) A method for screening a factor which proliferates the cell according to any one of (1) to (46), comprising using the cell.

(84) A method for screening a factor which induces the cell according to any one of (1) to (46) to differentiate into a cardiomyocyte, comprising using the cell.

(85) A method for screening a factor which immortalizes the cell according to any one of (1) to (46), comprising using the cell.

(86) A method for immortalizing the cell according to any one of (1) to (46), comprising expressing a telomerase in the cell.

(87) The method according to (86), wherein the telomerase comprises the amino acid sequence represented by SEQ ID NO:31.

(88) A therapeutic agent for a heart disease, comprising, as an active ingredient, the cell according to any one of (1) to (46) which has been immortalized by expressing a telomerase.

(89) The therapeutic agent according to (88), wherein the telomerase comprises the amino acid sequence represented by SEQ ID NO:31.

(90) A culture supernatant comprising the cell according to any one of (1) to (46).

(91) A method for inducing the cell according to any one of (1) to (46) to differentiate into a cardiomyocyte, comprising using the culture supernatant according to (90).

Therefore, according to a first aspect of this invention there is provided an isolated cell which has been isolated from bone marrow or umbilical blood, and which has the potential to differentiate into at least a cardiomyocyte, wherein the cell is CD14-negative, CD29-positive, CD44-positive and CD45-negative.

Other aspects of this invention which relate to this first aspect are as set out above.

The cells having the potential to differentiate into cardiomyocytes according to the present invention can be isolated from adult tissues such as bone marrow, muscle, brain, pancreas, liver and kidney or umbilical blood, and preferred examples include bone marrow and umbilical blood.

Any cell can be used as the pluripotent stem of the present invention, so long as it has the potential to

differentiate into cardiomyocytes and other cells. Preferable examples thereof include cells having the potential to differentiate into at least cardiomyocytes, adipocytes, skeletal muscle cells and osteoblasts; cells having the potential to differentiate into at least cardiomyocyte and vascular endothelial cells; cells having the potential to differentiate into at least cardiomyocytes, adipocytes, skeletal muscle cells, osteoblasts and vascular endothelial cells; and cells having the potential to differentiate into at least cardiomyocytes, adipocytes, skeletal muscle cells, vascular endothelial cells, osteoblasts, neural cells and hepatocytes.

Also, even if cells originally have the potential to differentiate into adipocytes, skeletal muscle cells and osteoblasts but do not have the potential to differentiate into cardiomyocytes, those cells to which the potential to differentiate into cardiomyocytes can be added by the following induction method or the like, are included in the invention.

The cells of the present invention having the potential to differentiate into cardiomyocytes include cells which are CD117-positive and CD140-positive. The cells which are CD117-positive and CD140-positive preferably cells which are CD34-positive, CD117-positive and CD140-positive, and cells which are CD34-negative, CD117-positive and CD140-positive; more preferably cells

which are CD144-positive, CD34-positive, CD117-positive and CD140-positive, cell which are CD144-negative, CD34-positive, CD117-positive and CD140-positive, cells which are CD144-positive, CD34-negative, CD117-positive and CD140-positive, and cells which are CD144-negative, CD34-negative, CD117-positive and CD140-positive; still more preferably cells which are CD34-positive, CD117-positive, CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD144-positive, CD140-positive, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative and CD44-positive, cells which are CD34-positive, CD117-positive, CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD144-negative, CD140-positive, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative and CD44-positive, cells which are CD34-negative, CD117-positive, CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD144-positive, CD140-positive, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative and CD44-positive, and cells which are CD34-positive, CD117-positive, CD14-negative, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD144-negative, CD140-positive, CD49b-negative, CD49d-negative, CD29-positive, CD54-negative, CD102-negative, CD106-negative and CD44-

positive. The cells which are CD117-positive and CD140-positive include mouse marrow multipotential stem cells, BMSC. Mouse bone marrow-derived pluripotent stem cells (BMSC) have been deposited on February 22, 2000, in National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology (Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan) as FERM BP-7043.

Examples of the cells which originally have the potential to differentiate into adipocytes, skeletal muscle cells and osteoblasts but do not have the potential to differentiate into cardiomyocytes, to which the potential to differentiate into heart muscle cells can be added by the following induction method or the like include cells which are CD117-negative and CD140-positive, preferably cells which are CD144-negative, CD34-negative, CD117-negative and CD140-positive, more preferably cells which are CD34-negative, CD117-negative, CD14-positive, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD144-negative, CD140-positive, CD49b-positive, CD49d-negative, CD29-positive, CD54-positive, CD102-negative, CD106-positive and CD44-positive. KUM2 cells can be exemplified as the cells which are CD34-negative, CD117-negative, CD14-positive, CD45-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD144-negative, CD140-positive, CD49b-positive,

CD49d-negative, CD29-positive, CD54-positive, CD102-negative, CD106-positive and CD44-positive.

As the species of the vital tissue or umbilical blood used in the invention, vertebrate animals, preferably warm blooded animals, and more preferably mammals such as mouse, rat, guinea pig, hamster, rabbit, cat, dog, sheep, pig, cattle, goat, monkey and human are used. Those derived from a human is preferred for human therapeutic use.

Any adult tissue or umbilical blood can be used, so long as it is derived from the above animal . In therapeutic use for the human body, it is preferred to employ those derived from humans.

Myocardial cells can be obtained by isolating cells having the potential to differentiate into cardiomyocytes from an adult tissue or umbilical blood of a mammal, such as mouse, rat or human, culturing these cells and then inducing the differentiation of cells having the potential to differentiate into cardiomyocytes.

The differentiation into not only cardiomyocytes but also vascular endothelial cells, smooth muscles, skeletal muscle cells, adipocytes, bones, cartilages, pancreatic endocrine cells, pancreatic exocrine cells, hepatocytes, glomerular cells, renal tubular cells, neurons, glial cells, oligodendrocytes, etc. can be induced using the pluripotent stem cell to obtain various cells.

Now, the present invention will be described in greater detail.

1. Isolation of cells having the potential to differentiate into cardiomyocytes

The cells having the potential to differentiate into cardiomyocytes according to the present invention can be isolated from any tissue (for example, an adult tissue, umbilical blood), so long as cells having the potential to differentiate into cardiomyocytes can be obtained. Next, a method for isolating cells having the potential to differentiate into cardiomyocytes from bone marrow will be illustrated.

(1) Method for isolating bone marrow cells having the potential to differentiate into cardiomyocytes

The method for obtaining human cells having the potential to differentiate into cardiomyocytes from bone marrow is not particularly limited, so long as it is a safe and efficient method. For example, the method described in S. E. Haynesworth, et al., *Bone*, 13: 81 (1992) can be employed.

Bone marrow puncture is conducted by sternal or iliac puncture. After skin disinfection of the part for puncture, a donor is subjected to local anesthesia. Particularly, subperiosteum is thoroughly anesthetized. The

inner tube of a bone marrow puncture needle is pulled out and a 10 ml syringe containing 5000 units of heparin is attached to the needle. A required amount, normally 10-20 ml, of the bone marrow fluid is quickly taken by suction and the puncture needle is removed, followed by pressure hemostasis for about 10 minutes. The obtained bone marrow fluid is centrifuged at $1000 \times g$ to recover bone marrow cells, which are then washed with PBS (phosphate buffered saline). After this centrifugation step is repeated twice, the obtained bone marrow cells are suspended in a cell culture medium such as α -MEM (α -modification of MEM), DMEM (Dulbecco's modified MEM) or IMDM (Isocove's modified Dulbeccos's medium) each containing 10% FBS (fetal bovine serum) to prepare a bone marrow cell suspension.

For the isolation of the bone marrow cells having the potential to differentiate into cardiomyocytes from the obtained bone marrow cell suspension, any method can be employed, so long as it is effective for removing other cells existing in the cell suspension such as hematocytes, hematopoietic stem cells, vascular stem cells and fibroblasts. For example, based on the method described in M.F. Pittenger et al., *Science*, 284: 143 (1999), the desired cells can be isolated by subjecting the cell suspension layered over Percoll having the density of 1.073 g/ml to centrifugation at $1100 \times g$ for 30 minutes, and the cells on the interface are recovered. Furthermore, a bone

marrow cell mixture containing the cells having the potential to differentiate into cardiomyocytes can be obtained by mixing the above cell suspension with an equal amount of Percoll solution diluted to 9/10 with 10x PBS, followed by centrifugation at $20000 \times g$ for 30 minutes, and recovering the fraction having the density of 1.075-1.060.

The thus obtained bone marrow cell mixture containing the bone marrow cells having the potential to differentiate into cardiomyocytes is diluted into single cell using 96-well culture plates to prepare a number of clones respectively derived from single cells. The clones having the potential to differentiate into cardiomyocyte can be selected by the observation of spontaneously beating cells generated by the treatment to induce cardiomyocytes from the cells having the potential to differentiate into cardiomyocytes described below.

Rat- or mouse-derived bone marrow cells having the potential to differentiate into cardiomyocytes can be obtained, for example, in the following manner. A rat or a mouse is sacrificed by cervical dislocation and thoroughly disinfected with 70% ethanol. After the skin on the femur and quadriceps femuris are excised, the femur is put out of the knee joint with scissors and the muscle on the back side of the femur is removed. Then, the femur is put out of the hip joint with scissors and taken out. After the muscle on the femur is removed with scissors as completely

as possible, the femur is cut at both ends using scissors. A needle having a size appropriate for the thickness of the bone is attached to a 2.5 ml syringe containing about 1.5 ml of a cell culture medium such as α -MEM, DMEM or IMDM each containing 10% FBS followed by injecting into the pore of femur. The needle of the syringe is put into the femur from the cut end of the knee joint side and the culture medium is injected into bone marrow, whereby bone marrow cells are pressed out of the bone from the cut end of the hip joint side. The thus obtained bone marrow cells are suspended in a culture medium by pipetting. The bone marrow cells having the potential to differentiate into cardiomyocytes can be isolated from the resulting cell suspension in the same manner as in the above isolation of the human bone marrow cells.

(2) Method for isolating cells having the potential to differentiate into cardiomyocytes from tissue other than bone marrow

According to the separation method using antibodies as described in 12 hereinafter, cells having the potential to differentiate into cardiomyocytes can be obtained from tissues other than bone marrow.

Preferred examples of the tissues other than bone marrow include umbilical blood. More specifically, it can be isolated in the following method.

First, umbilical blood is separated from the cord, followed by addition of heparin to give a final concentration of 500 units/ml. After thoroughly mixing, cells are separated from the umbilical blood by centrifugation and re-suspended in a cell culture medium, such as α -MEM (α -modified MEM), DMEM (Dulbecco's modified MEM) or IMDM (Isocove's modified Dulbecco's medium), each containing 10% FBS. From the cell suspension thus obtained, cells having the potential to differentiate into cardiomyocytes can be separated using the antibodies described below.

2. Methods for culturing the cells having the potential to differentiate into cardiomyocytes

The cells having the potential to differentiate into cardiomyocytes isolated by the methods described in the above 1 can be usually cultured using media of known compositions (*Technical Standard of Tissue Culture*, Third Edition, Asakura Shoten (1996)). Preferred media are cell culture media such as α -MEM, DMEM and IMDM supplemented with a serum such as 5-20% bovine serum. Culturing can be carried out under any conditions suitable for cell culture, but is preferably carried out at a temperature of 33-37°C in an incubator filled with 5-10% carbon dioxide gas. It is preferred to culture the cells having the potential to differentiate into cardiomyocytes in a plastic culture dish

used for ordinary tissue culture so that the grown cells adhere to the dish. When cells become confluent on the dish, the medium is removed and a trypsin-EDTA solution is added to suspend the cells therein. The suspended cells may be washed with PBS or a medium for culturing the cells, diluted 5-20 times with the medium and then added to another culture dish for subculture.

3. Methods for inducing cardiomyocytes from cells having the potential to differentiate into cardiomyocytes

The methods for inducing cardiomyocytes from the cells having the potential to differentiate into cardiomyocytes include the following: (1) induction of differentiation by the treatment with a DNA-demethylating agent, (2) induction of differentiation using a factor which is expressed in the cardiogenesis region of a fetus or a factor which controls differentiation into cardiomyocytes in the cardiogenesis stage of a fetus, and (3) induction of differentiation using a culture supernatant of the cells having the potential to differentiate into cardiomyocytes or cardiomyocytes differentiated from the cells. Cardiomyocytes can be induced from the cells having the potential to differentiate into cardiomyocytes using such a method alone or in combination. Also, according to these methods, even mesenchymal cells which originally do not have the

potential to differentiate into cardiomyocytes can be differentiated into cells having the potential to differentiate into cardiomyocytes, and cardiomyocytes can be induced.

Any DNA-demethylating agent can be used, so long as it is a compound which causes demethylation of DNA. Suitable DNA-demethylating agents include demethylase which is an enzyme which specifically removes the methylation of the cytosine residue in the GpC sequence in a chromosomal DNA, 5-azacytidine (hereinafter referred to as "5-aza-C") and DMSO (dimethyl sulfoxide). Examples of the demethylase enzymes include demethylase having the amino acid sequence represented by SEQ ID NO:1 (*Nature*, 397: 579-583 (1999)). Differentiation can be induced by the treatment with a DNA-demethylating agent, for example, in the following manner.

The cells having the potential to differentiate into cardiomyocytes are cultured in the presence of 3 $\mu\text{mol/l}$ to 10 $\mu\text{mol/l}$ of 5-aza-C for 24 hours. After 5-aza-C is removed by replacing the culture supernatant with a fresh medium, the cells are cultured for further 2-3 weeks to obtain cardiomyocytes. The cardiomyocytes produced by culturing for 2-3 weeks are mainly sinus node cells, but culturing for more than 4 weeks induces differentiation into ventricular cardiomyocytes.

Examples of the factors which are expressed in the cardiogenesis region of a fetus and the factors which act

on differentiation into cardiomyocytes in the cardiogenesis stage of a fetus include cytokines, vitamins, adhesion molecules and transcription factors.

Any cytokine can be used, so long as it stimulates the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes in the cardiogenesis stage.

The examples include platelet-derived growth factor (hereinafter referred to as "PDGF"), fibroblast growth factor 8 (FGF8), endothelin 1 (ET1), midkine, and bone morphogenic protein 4 (BMP4). Preferred examples of the PDGF include PDGF A, PDGF B, PDGF C and the like, and specific examples include those the amino acid sequences represented by SEQ ID NOS:3 and 5. Preferred examples of the FGF8, ET1, midkine, BMP4 include the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively. The cytokine can be used, e.g., at a concentration of 10 to 40 ng/ml.

It is also possible to stimulate the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes into cardiomyocytes in the cardiogenesis stage using an inhibitor against a

cytokine which suppresses the cardiomyogenic differentiation.

The cytokines which suppress the cardiomyogenic differentiation include fibroblast growth factor-2 (hereinafter referred to as "FGF-2"), specifically, FGF-2 having the amino acid sequence represented by SEQ ID NO:7 or 8.

The inhibitors against the cytokines which suppress the cardiomyogenic differentiation include substances which inhibit the signal transduction of the cytokines, such as antibodies and low molecular weight compounds which neutralize the cytokines activities.

Any vitamin can be used, so long as it stimulates the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes in the cardiogenesis stage. Retinoic acid can be used, e.g., at a concentration of 10^{-9} M.

Any adhesion molecule can be used, so long as it is expressed in the cardiogenesis region in the cardiogenesis stage. Examples include extracellular matrices such as gelatin, laminin, collagen, fibronectin and the like. For example, the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes can be stimulated by culturing the cells on a culture dish coated with fibronectin.

Examples of the transcription factors include a homeobox-type transcription factor, Nkx2.5/Csx (SEQ ID NO:9, amino acid sequence; SEQ ID NO:10, nucleotide sequence); a zinc finger-type transcription factor belonging to the GATA family, GATA4 (SEQ ID NO:11, amino acid sequence; SEQ ID NO:12, nucleotide sequence); transcription factors belonging to the myocyte enhance factor-2 (MEF-2) family, MEF-2A (SEQ ID NO:13, amino acid sequence; SEQ ID NO:14, nucleotide sequence), MEF-2B (SEQ ID NO:15, amino acid sequence; SEQ ID NO:16, nucleotide sequence), MEF-2C (SEQ ID NO:17, amino acid sequence; SEQ ID NO:18, nucleotide sequence) and MEF-2D (SEQ ID NO:19, amino acid sequence; SEQ ID NO:20, nucleotide sequence); transcription factors belonging to the basic helix loop helix-type transcription factors, dHAND (SEQ ID NO:21, amino acid sequence; SEQ ID NO:22, nucleotide sequence) and eHAND (SEQ ID NO:23, amino acid sequence; SEQ ID NO:24, nucleotide sequence); and transcription factors belonging to the family of TEA-DNA binding-type transcription factors, TEF-1 (SEQ ID NO:25, amino acid sequence; SEQ ID NO:26, nucleotide sequence), TEF-3 (SEQ ID NO:27, amino acid sequence; SEQ ID NO:28, nucleotide sequence) and TEF-5 (SEQ ID NO:29, amino acid sequence; SEQ ID NO:30, nucleotide sequence).

The cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes can be induced by introducing DNA encoding one or

combination of the above-described factors into the cells and expressing the DNA therein.

It is also possible to induce the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes by culturing them using a culture dish coated with an extracellular matrix obtained from spontaneously beating cardiomyocytes, co-culturing with spontaneously beating cardiomyocytes or adding a culture supernatant of spontaneously beating cardiomyocytes.

Furthermore, a factor which induces differentiation of cardiomyocytes which are obtained by the method described in 4 below (hereinafter referred to as "the cardiomyogenic differentiation-inducing factor") can also be used in inducing the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes.

4. Methods for obtaining cardiomyogenic differentiation-inducing factors

A cardiomyogenic differentiation-inducing factor can be obtained by adding various protease inhibitors to a culture supernatant of spontaneously beating cardiomyocytes, followed by combinations of treatments, such as dialysis, salting-out and chromatography.

Genes encoding such cardiomyogenic differentiation-inducing factors can be obtained by determining partial

amino acid sequences of these factors using a microsequencer followed by screening a cDNA library prepared from the spontaneously beating cells using DNA probes designed based on the determined amino acid sequences.

5. Therapeutic agents for cardiac regeneration and therapeutic agents for heart diseases comprising cells having the potential to differentiate into cardiomyocytes

The cells having the potential to differentiate into cardiomyocytes according to the present invention can be used as therapeutic agents for cardiac regeneration or for heart diseases.

The heart diseases include myocardial infarction, ischemic heart disease, congestive heart failure, arrhythmia, hypertrophic cardiomyopathy, dilated cardiomyopathy, myocarditis and valvular disease.

The agents for cardiac regeneration contain the cells having the potential to differentiate into cardiomyocytes of high purity which cells have been proliferated *in vitro* according to the position and size of the damaged part of the heart. The preferred cells having the potential to differentiate into cardiomyocytes are those which can be induced to differentiate into various cells constituting the heart such as endocardial

endothelial cells, cushion cells, ventricular cardiomyocytes, atrial cardiomyocytes and sinus node cells.

The therapeutic agents can be prepared by purifying the cells having the potential to differentiate into cardiomyocytes from the bone marrow fluid taken from myocardial infarction patients according to the above-described density gradient centrifugation, the panning method (*J. Immunol.*, 141(8): 2797-800 (1988)) or the FACS method (*Int. Immunol.*, 275-83 (1998)) using the antibodies described below which specifically recognize the cells having the potential to differentiate into cardiomyocytes, or a method for constructing a reporter system using the promoter of a gene specifically expressed in the cell having the potential to differentiate into cardiomyocytes.

The therapeutic agents include cardiomyocytes derived from the cells having the potential to differentiate into cardiomyocytes using the myocardium-forming agent described below as well as the cells having the potential to differentiate into cardiomyocytes which are obtained by activating the division potential of the bone marrow cells taken from the bone marrow of aged persons by utilizing the immortalization method described below.

The purity of the therapeutic agents prepared according to the above methods can be tested by the FACS method combined with the antibodies which specifically

recognize the cells having the potential to differentiate into cardiomyocytes.

The therapeutic agents can be transported to the damaged parts by a method using a catheter or the like. For example, in the case of ischemic heart disease, the therapeutic agents are transported according to the following procedure. Since the cardiomyocytes damaged by ischemic heart disease exist downstream of vascular stricture, it is necessary to locate the vascular stricture by coronary arteriography (*Illustrated Pathological Internal Medical Course Circulatory Organ*, 1, MEDICAL VIEW, 1993) prior to the injection of the above cells. Organic stricture is classified as concentric stricture, eccentric stricture or multiple mural asymmetry according to type of stricture, and eccentric stricture is further classified into two types, i.e. type I and type II. It is known that the types of stricture are related to the course and prognosis of angina; for instance, eccentric stricture of type II and multiple mural asymmetry are often observed in unstable angina which is liable to shift into myocardial infarction. In cases where blood vessels are completely strictured, there is the possibility that the injected cells can not reach the damaged parts. In such cases, the strictured parts must be reopened by means of percutaneous transluminal coronary angioplasty (PTCA), thrombolytic treatment or the like prior to the cell injection. The

type of the cells to be injected such as ventricular or atrial can be selected according to the position of the damaged cardiomyocytes. The insertion of a catheter can be performed by the Sones method (*Illustrated Pathological Internal Medical Course Circulatory Organ*, 1, MEDICAL VIEW, 1993) through the artery of the right upper arm or by the Jundkins method (*Illustrated Pathological Internal Medical Course Circulatory Organ*, 1, MEDICAL VIEW, 1993) through the femoral artery.

6. Myocardium-forming agents

The myocardium-forming agents according to the present invention comprise, as an active ingredient, at least one cardiomyogenic differentiation-inducing factor selected from the group consisting of a chromosomal DNA-demethylating agent, a factor which is expressed in the cardiogenesis region of a fetus, and a factor which acts on differentiation into cardiomyocytes in the cardiogenesis stage of a fetus, and are capable of inducing the bone marrow-derived cells to differentiate into cardiomyocytes.

Examples of the cardiomyogenic differentiation-inducing factors include cytokines, vitamins, adhesion molecules and transcription factors.

Any cytokine can be used, so long as it stimulates the cardiomyogenic differentiation of the cells having the

potential to differentiate into cardiomyocytes in the cardiogenesis stage.

For example, PDGF, FGF-8, endotherin 1 (ET1), Midkine and Bone Marrow Protein 4 (BMP4) can be used. Preferable examples of the PDGF, FGF8, ET1, Midkine, BMP4 include those the amino acid sequences represented by SEQ ID NOS:3 and 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively. The cytokine can be used, e.g., at a concentration of 10 to 40 ng/ml.

Any vitamin can be used, so long as it stimulates the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes in the cardiogenesis stage. Retinoic acid can be used, e.g., at a concentration of 10^{-9} M.

Any adhesion molecule can be used so far as it is expressed in the cardiogenesis region in the cardiogenesis stage. Examples include gelatin, laminin, collagen, fibronectin and the like. For example, the cardiomyogenic differentiation of the cells having the potential to differentiate into cardiomyocytes can be stimulated by culturing the cells in a culture dish coated with fibronectin.

Examples of the transcription factors include a homeobox-type transcription factor, Nkx2.5/Csx (SEQ ID NO:9, amino acid sequence; SEQ ID NO:10, nucleotide sequence); a zinc finger-type transcription factor belonging to the GATA family, GATA4 (SEQ ID NO:11, amino acid sequence; SEQ ID NO:12, nucleotide sequence); transcription factors belonging to the myocyte enhancer factor-2 (MEF-2) family, MEF-2A (SEQ ID NO:13, amino acid sequence; SEQ ID NO:14, nucleotide sequence), MEF-2B (SEQ ID NO:15, amino acid sequence; SEQ ID NO:16, nucleotide acid sequence), MEF-2C (SEQ ID NO:17, amino acid sequence; SEQ ID NO:18, nucleotide sequence) and MED-2D (SEQ ID NO:19, amino acid sequence; SEQ ID NO:20, nucleotide sequence); transcription factors belonging to the basic helix loop helix-type transcription factors, dHAND (SEQ ID NO:21, amino acid sequence; SEQ ID NO:22, nucleotide sequence), eHAND (SEQ ID NO:23, amino acid sequence; SEQ ID NO:24, nucleotide sequence) and MesP1 (SEQ ID NO:61, amino acid sequence; SEQ ID NO:62, nucleotide sequence); and transcription factors belonging to the family of TEA-DNA binding-type transcription factors, TEF-1 (SEQ ID NO:25, amino acid sequence; SEQ ID NO:26, nucleotide sequence), TEF-3 (SEQ ID NO:27, amino acid sequence; SEQ ID NO:28, nucleotide sequence) and TEF-5 (SEQ ID NO:29, amino acid sequence; SEQ ID NO:30, nucleotide sequence).

The myocardium-forming agents can contain, as a main component, either a gene encoding a cardiomyogenic differentiation-inducing factor or a protein which is a cardiomyogenic differentiation-inducing factor itself.

(1) Myocardium-forming agent containing gene as main Component

Methods for preparing the myocardium-forming agents of the present invention which comprise, as a main component, a gene encoding a cardiomyogenic differentiation-inducing factor are described below.

First, a DNA fragment or the full length cDNA of a gene encoding a cardiomyogenic differentiation-inducing factor is inserted downstream of a promoter in a virus vector plasmid to construct a recombinant virus vector plasmid.

Then, the obtained recombinant virus vector plasmid is introduced into a packaging cell which is suitable for the virus vector plasmid.

The recombinant virus vector plasmid lacks at least one of the genes encoding the proteins necessary for the packaging of a virus. As the packaging cell, any cell can be used so far as it can supply the protein encoded by the lacking gene. Suitable packaging cells include HEK293 cell derived from human kidney and mouse fibroblast NIH3T3.

Examples of the proteins supplied by the packaging cells include proteins, such as gag, pol and env, derived from mouse retroviruses for retrovirus vectors; proteins, such as gag, pol, env, vpr, vpu, vif, tat, rev and nef, derived from HIV viruses for lentivirus vectors; proteins, such as ElA and ElB, derived from adenoviruses for adenovirus vectors; and proteins, such as Rep(p5, p19, p40) and Vp(Cap), for adeno-associated viruses.

The virus vector plasmids that can be employed are those capable of producing a recombinant virus in the above packaging cells and comprising a promoter at a position appropriate for the transcription of a wild-type gene corresponding to the causative gene of a congenital genetic heart disease in cardiomyocytes.

Suitable virus vector plasmids include MFG (*Proc. Natl. Acad. Sci. USA*, 92: 6733-6737 (1995)), pBabePuro (*Nucleic Acids Research*, 18: 3587-3596 (1990)), LL-CG, CL-CG, CS-CG and CLG (*Journal of Virology*, 72: 8150-8157 (1998)) and pAdex1 (*Nucleic Acids Res.*, 23: 3816-3812 (1995)).

Any promoter can be used as long as it can be expressed in human tissues. Examples of suitable promoters are the promoter of IE (immediate early) gene of cytomegalovirus (human CMV), SV40 early promoter, the promoter of a retrovirus, metallothionein promoter, heat shock protein promoter and SR α promoter. The enhancer of

IE gene of human CMV may be used in combination with the promoter. It is possible to express the desired gene specifically in cardiomyocytes using a promoter of a gene specifically expressed in cardiomyocytes such as Nkx2.5/Csx gene.

A recombinant virus vector can be produced by introducing the above recombinant virus vector plasmid into the above packaging cell. Introduction of the virus vector plasmid into the packaging cell can be carried out, for example, by the calcium phosphate method (Japanese Published Unexamined Patent Application No. 227075/90) or the lipofection method (*Proc. Natl. Acad. Sci. USA*, 84: 7413 (1987)).

The above recombinant virus vector can be formulated into myocardium-forming agents by admixture with a carrier used in pharmaceutical compositions for gene therapy (*Nature Genet.*, 8: 42 (1994)). Any carrier can be used so long as it is usually used in injections. Suitable carriers include distilled water, salt solutions of sodium chloride or mixtures of sodium chloride and inorganic salts, solutions of mannitol, lactose, dextran, glucose, etc., solutions of amino acids such as glycine and arginine, and mixtures of organic acid solutions or salt solutions and a glucose solution. Injections may be prepared in the form of solutions, suspensions or dispersed solutions according to conventional methods using the above carriers as well as

auxiliaries, for example, osmotic pressure adjusting agents, pH adjusting agents, vegetable oils such as sesame oil and soybean oil, lecithin, and surfactants such as nonionic surfactants. If desired, the injections may be prepared in the form of powdered or freeze-dried preparations which are dissolved in a solvent before each use. The myocardium-forming agents in the form of liquid preparations can be used as such for gene therapy, and those in the form of solid preparations are dissolved, immediately before use, in the above carriers which are sterilized if necessary. Administration of the myocardium-forming agents is made locally using a catheter or the like so that the agents can be absorbed into the myocardium of a patient.

The cells having the potential to differentiate into cardiomyocytes infected with the above recombinant virus vector *in vitro* can also be formulated into the above myocardium-forming agents and administered to a patient. Furthermore, the recombinant virus vector can be directly administered to the diseased part of a patient.

(2) Myocardium-forming agent containing protein as main component

Methods for preparing the myocardium-forming agents of the present invention which contains as a main component, a protein which is a cardiomyogenic differentiation-inducing factor are described below.

On the basis of the full length cDNA encoding a cardiomyogenic differentiation-inducing factor, if necessary, a DNA fragment having an appropriate length containing a region encoding the protein is prepared.

The prepared DNA fragment or the full length cDNA is inserted downstream of a promoter in an expression vector to construct a recombinant expression vector for the protein.

Then, the recombinant expression vector is introduced into a host cell suited for the expression vector.

Any cell can be used so long as it is capable of expressing the desired gene products. Examples of the host cells include bacteria belonging to the genus *Escherichia*, the genus *Serratia*, the genus *Corynebacterium*, the genus *Brevibacterium*, the genus *Pseudomonas*, the genus *Bacillus* and the genus *Microbacterium*, yeasts belonging to the genus *Kluyveromyces*, the genus *Saccharomyces*, the genus *Shizosaccharomyces*, the genus *Trichosporon* and the genus *Schwanniomyces*, animal cells and insect cells.

The expression vectors that can be employed are those capable of autonomous replication or integration into chromosome in the above host cells and containing a promoter at a position suitable for the transcription of a gene of a cardiomyogenic differentiation-inducing factor.

When bacteria are used as the host cell, it is preferred that the recombinant expression vector for a gene encoding a cardiomyogenic differentiation-inducing factor is a recombinant vector which is capable of autonomous replication in the bacterial cell and which comprises a promoter, a ribosome binding sequence, a DNA encoding a protein which can induce cardiomyogenic differentiation, and a transcription termination sequence. The vector can further comprise a gene regulating the promoter.

Examples of suitable expression vectors include pBTrp2, pBTac1 and pBTac2 (manufactured by Boehringer Mannheim), pKK233-2 (manufactured by Amersham Pharmacia Biotech), pSE280 (manufactured by Invitrogen), pGEMEX-1 (manufactured by Promega), pQE-8 (manufactured by QIAGEN), pKYP10 (Japanese Published Unexamined Patent Application No. 110600/83), pKYP200 (*Agricultural Biological Chemistry*, 48: 669 (1984)), pLSA1 (*Agric. Biol. Chem.*, 53: 277 (1989)), pGEL1 (*Proc. Natl. Acad. Sci. USA*, 82: 4306 (1985)), pBluescript II SK (-) (manufactured by Stratagene), pGEX (manufactured by Amersham Pharmacia Biotech), pET-3 (manufactured by Novagen), pTerm2 (U.S. Patents 4,686,191, 4,939,094 and 5,160,735), and pSupex, pUB110, pTP5, pC194 and pEG400 (*J. Bacteriol.*, 172: 2392 (1990)).

It is preferred to use a plasmid in which the distance between the Shine-Dalgarno sequence (ribosome

binding sequence) and the initiation codon is adjusted to a suitable length (e.g., 6-18 bases).

Any promoter can be used so long as it can be expressed in the host cell. For example, promoters derived from *Escherichia coli* or a phage, such as *trp* promoter (P_{trp}), *lac* promoter (P_{lac}), P_L promoter, P_R promoter and T7 promoter, SP01 promoter, SP02 promoter and *penP* promoter can be used. Artificially modified promoters such as a promoter in which two P_{trp} are combined in tandem ($P_{trp \times 2}$), *tac* promoter, *letI* promoter (Gene, 44: 29 (1986)) and *lacT7* promoter can also be used.

The yield of the desired protein can be improved by replacing a nucleotide in the nucleotide sequence of the protein-encoding region in the gene of the cardiomyogenic differentiation-inducing factor of the present invention so as to make a codon most suitable for the expression in a host cell.

The transcription termination sequence is not essential for the expression of the gene encoding the cardiomyogenic differentiation-inducing factor of the present invention, but it is preferred that the transcription termination sequence is located immediately downstream of the structural gene.

Examples of suitable host cells are cells of microorganisms belonging to the genus *Escherichia*, the genus *Serratia*, the genus *Corynebacterium*, the genus

Brevibacterium, the genus *Pseudomonas*, the genus *Bacillus* and the genus *Microbacterium*, specifically, *Escherichia coli* XL1-Blue, *Escherichia coli* XL2-Blue, *Escherichia coli* DH1, *Escherichia coli* MC1000, *Escherichia coli* KY3276, *Escherichia coli* W1485, *Escherichia coli* JM109, *Escherichia coli* HB101, *Escherichia coli* No. 49, *Escherichia coli* W3110, *Escherichia coli* NY49, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Brevibacterium ammoniagenes*, *Brevibacterium immariophilum* ATCC 14068, *Brevibacterium saccharolyticum* ATCC 14066, *Corynebacterium glutamicum* ATCC 13032, *Corynebacterium glutamicum* ATCC 14067, *Corynebacterium glutamicum* ATCC 13869, *Corynebacterium acetoacidophilum* ATCC 13870, *Microbacterium ammoniaphilum* ATCC 15354 and *Pseudomonas* sp. D-0110.

Introduction of the recombinant vector can be carried out by any of the methods for introducing DNA into the above host cells, for example, the method using calcium ion (*Proc. Natl. Acad. Sci. USA*, 69: 2110 (1972)), the protoplast method (Japanese Published Unexamined Patent Application No. 248394/88) and the methods described in *Gene*, 17: 107 (1982) and *Molecular & General Genetics*, 168: 111 (1979).

When yeast is used as the host cell, YEp13 (ATCC 37115), YEp24 (ATCC 37051), YCp50 (ATCC 37419), pHS19, pHS15, etc. can be used as the expression vector.

Any promoter can be used, so long as it can be expressed in the yeast. Suitable promoters include PH05 promoter, PGK promoter, GAP promoter, ADH promoter, gal 1 promoter, gal 10 promoter, heat shock protein promoter, MF α 1 promoter and CUP 1 promoter.

Examples of suitable host cells include cells of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Trichosporon pullulans* and *Schwanniomyces alluvius*.

Introduction of the recombinant vector can be carried out by any of the methods for introducing DNA into yeast cells, for example, electroporation (*Methods. Enzymol.*, 194: 182 (1990)), the spheroplast method (*Proc. Natl. Acad. Sci. USA*, 75: 1929 (1978)) and the lithium acetate method (*J. Bacteriol.*, 153: 163 (1983), *Proc. Natl. Acad. Sci. USA*, 75: 1929 (1978)).

When an animal cell is used as the host cell, pCDNAI (manufactured by Invitrogen), pCDM8 (manufactured by Invitrogen), pAGE107 (Japanese Published Unexamined Patent Application No. 22979/91, *Cytotechnology*, 3: 133 (1990)), pAS3-3 (Japanese Published Unexamined Patent Application No. 227075/90), pCDM8 (*Nature*, 329: 840 (1987)), pCDNAI/Amp (manufactured by Invitrogen), pREP4 (manufactured by Invitrogen), pAGE103 (*J. Biochem.* 101: 1307 (1987)), pAGE210, etc. can be used as the expression vector.

As the promoter, any promoters capable of expression in animal cells can be used. Suitable promoters include the promoter of IE (immediate early) gene of cytomegalovirus (human CMV), SV40 early promoter, the promoter of a retrovirus, metallothionein promoter, heat shock protein promoter and SR α promoter. The enhancer of IE gene of human CMV may be used in combination with the promoter.

Examples of suitable host cells are human Namalwa cell, monkey COS cell, Chinese hamster CHO cell and HBT5637 (Japanese Published Unexamined Patent Application No. 299/88).

Introduction of the recombinant vector can be carried out by any of the methods for introducing DNA into animal cells, for example, electroporation method (*Cytotechnology*, 3: 133 (1990)), the calcium phosphate method (Japanese Published Unexamined Patent Application No. 227075/90), and lipofection method (*Proc. Natl. Acad. Sci. USA*, 84: 7413 (1987), *Virology*, 52: 456 (1973)). A transformant can be obtained and cultured according to the methods described in Japanese Published Unexamined Patent Application Nos. 227075/90 and 257891/90.

When an insect cell is used as the host cell, the protein can be expressed using the methods described in *Baculovirus Expression Vectors, A Laboratory Manual*, W.H. Freeman and Company, New York (1992), *Current Protocols in*

Molecular Biology, Supplement 1-38 (1987-1997), *Bio/Technology*, 6: 47 (1988), etc.

Specifically, the recombinant gene transfection vector and a baculovirus are cotransfected into an insect cell to obtain a recombinant virus in the culture supernatant of the insect cell, and then an insect cell is infected with the recombinant virus to express the protein.

Examples of the gene transfection vectors suitable for use in this method are pVL1392, pVL1393 and pBlueBacIII (manufactured by Invitrogen).

Examples of the baculovirus include *Autographa californica* nuclear polyhedrosis virus with which an insect belonging to the family *Barathra* is infected.

Examples of the insect cells include Sf9 and Sf21 (*Baculovirus Expression Vectors, A Laboratory Manual*, W.H. Freeman and Company, New York (1992)), which are ovary cells of *Spodoptera frugiperda*, and High 5 (manufactured by Invitrogen), which is an ovary cell of *Trichoplusia ni*.

Cotransfection of the recombinant gene transfection vector and the baculovirus into an insect cell for the preparation of the recombinant virus can be carried out by the calcium phosphate method (Japanese Published Unexamined Patent Application No. 227075/90), the lipofection method (*Proc. Natl. Acad. Sci. USA*, 84: 7413 (1987)), etc.

Expression of the gene can be carried out not only by direct expression but also by secretory production,

fused protein expression, etc. according to the methods described in *Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press (1989) (hereinafter referred to as "*Molecular Cloning, A Laboratory Manual*, 2nd ed.") etc.

When the gene is expressed in yeast, an animal cell or an insect cell, a glycoprotein or glycosylated protein can be obtained.

The protein as the cardiomyogenic differentiation-inducing factor can be produced by culturing the transformant carrying the recombinant DNA containing the DNA encoding the protein as the cardiomyogenic differentiation-inducing factor in a medium, allowing the protein to accumulate in the culture, and recovering the protein from the culture.

Culturing of the transformant for the production of the protein as the cardiomyogenic differentiation-inducing factor can be carried out by conventional methods for culturing the host cell of the transformant.

For the culturing of the transformant prepared using a procaryotic cell such as *E. coli* or a eucaryotic cell such as yeast as the host cell, any of natural media and synthetic media can be used, so long as it is a medium suitable for efficient culturing of the transformant which contains a carbon source, a nitrogen source, an inorganic substance, etc. which can be assimilated by the host used.

Any carbon source can be used, so long as it can be assimilated by the host. Examples of suitable carbon sources include carbohydrates such as glucose, fructose, sucrose, molasses containing them, starch and starch hydrolyzate; organic acids such as acetic acid and propionic acid; and alcohols such as ethanol and propanol.

Examples of the nitrogen sources include ammonia, ammonium salts of inorganic or organic acids such as ammonium chloride, ammonium sulfate, ammonium acetate and ammonium phosphate, and other nitrogen-containing compounds can be used as well as peptone, meat extract, yeast extract, corn steep liquor, casein hydrolyzate, soybean cake, soybean cake hydrolyzate, and various fermented cells and digested products thereof.

Examples of the inorganic substances include potassium dihydrogenphosphate, dipotassium hydrogenphosphate, magnesium phosphate, magnesium sulfate, sodium chloride, ferrous sulfate, manganese sulfate, copper sulfate and calcium carbonate.

Culturing is usually carried out under aerobic conditions, for example, by shaking culture or submerged spinner culture under aeration, at 15-40°C for 16 hours to 7 days. The pH is maintained at 3.0-9.0 during the culturing. The pH is adjusted using an organic or inorganic acid, an alkali solution, urea, calcium carbonate, ammonia, etc.

If necessary, antibiotics, such as ampicillin and tetracycline, can be added to the medium during the culturing.

When a microorganism transformed with an expression vector comprising an inducible promoter is cultured, an inducer may be added to the medium, if necessary. For example, in the case of a microorganism transformed with an expression vector containing *lac* promoter, isopropyl- β -D-thiogalactopyranoside (IPTG) or the like can be added to the medium; and in the case of a microorganism transformed with an expression vector containing *trp* promoter, indoleacrylic acid (IAA) or the like can be added.

For the culturing of the transformant prepared using an animal cell as the host cell, generally used media such as RPMI1640 medium (*The Journal of the American Medical Association*, 199: 519 (1967)), Eagles's MEM (*Science*, 122: 501 (1952)), Dulbecco's modified MEM (*Virology*, 8: 396 (1959)) and 199 medium (*Proceeding of the Society for the Biological Medicine*, 73: 1 (1950)), media prepared by adding fetal calf serum to these media, etc. can be used as the medium.

Culturing is usually carried out at pH 6-8 at 30-40°C for 1-7 days in the presence of 5% CO₂.

If necessary, antibiotics, such as kanamycin and penicillin, can be added to the medium during the culturing.

For the culturing of the transformant prepared using an insect cell as the host cell, generally used media such as TNM-FH medium (manufactured by Pharmingen), Sf-900II SFM medium (manufactured by Life Technologies), ExCell 400 and ExCell 405 (manufactured by JRH Biosciences) and Grace's Insect Medium (Grace, T.C.C., *Nature*, 195: 788 (1962)) can be used as the medium.

Culturing is usually carried out at pH 6-7 at 25-30°C for 1-5 days.

If necessary, antibiotics, such as gentamicin, can be added to the medium during the culturing.

The protein as the cardiomyogenic differentiation-inducing factor can be isolated and purified from the culture of the transformant by conventional methods for isolating and purifying proteins.

For example, when the protein as the cardiomyogenic differentiation-inducing factor is expressed in a soluble form in cells, the isolation and purification can be carried out in the following manner. After the completion of culturing, the cells are recovered from the culture by centrifugation and suspended in an aqueous buffer, followed by disruption using an ultrasonic disrupter, a French press, a Manton Gaulin homogenizer, a Dyno Mill, etc. to obtain a cell-free extract. The cell-free extract is centrifuged, and a purified protein preparation can be produced from the obtained supernatant using ordinary means for isolation and

purification of proteins, for example, extraction with a solvent, salting-out with ammonium sulfate, etc., desalting, precipitation with an organic solvent, anion exchange chromatography using resins such as diethylaminoethyl (DEAE)-Sephacrose and DIAION HPA-75 (Mitsubishi Chemical Corporation), cation exchange chromatography using resins such as S-Sephacrose FF (manufactured by Amersham Pharmacia Biotech), hydrophobic chromatography using resins such as butyl Sepharose and phenyl Sepharose, gel filtration using a molecular sieve, affinity chromatography, chromatofocusing, and electrophoresis such as isoelectric focusing, alone or in combination.

When the protein is expressed as an insoluble substance in cells, the cells are separated and disrupted, followed by centrifugation to recover the insoluble substance of the protein as a precipitate fraction.

The recovered insoluble substance of the protein is solubilized with a protein-denaturing agent. The solubilized protein solution is diluted or dialyzed to lower the concentration of the protein-denaturing agent therein, thereby restoring the normal tertiary structure of the protein, followed by the same isolation and purification steps as described above to obtain a purified protein preparation.

When the protein is the cardiomyogenic differentiation-inducing factor or its derivatives, such as

a glycosylated protein, are extracellularly secreted, they can be recovered from the culture supernatant. That is, the culture is treated by means such as centrifugation and the obtained culture supernatant is subjected to the same isolation and purification steps as mentioned above to obtain a purified protein preparation.

The thus obtained proteins include the proteins having the amino acid sequences represented by SEQ ID NOS:5, 6, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30.

The proteins expressed by the above methods can also be produced by chemical synthetic methods such as the Fmoc method (the fluorenylmethyloxycarbonyl method) and the tBoc method (the t-butyloxycarbonyl method). Furthermore, the proteins can be synthesized using peptide synthesizers (for example, manufactured by Advanced ChemTech, Perkin-Elmer, Amersham Pharmacia Biotech, Protein Technology Instrument, Synthecell-Vega, PerSeptive, Shimadzu Corporation, etc.).

The protein which can induce cardiomyogenic differentiation can be formulated into myocardium-forming agents and administered in the same manner as in the above (1).

7. Application to therapy of congenital genetic disease

In some of the diseases leading to heart failure, the deficiency of an essential protein due to the mutation

of a single gene causes heart failure. Examples of such diseases are familial hypertrophic cardiomyopathy, Fabri disease, QT elongation syndrome, Marfan syndrome, aortic stenosis, mitochondria cardiomyopathy and Duchenne muscular dystrophy. These diseases are known to be caused by the abnormality in the genes of myosin, troponin, tropomyosin, potential-dependent Na channel, K channel, fibrin, elastin, mitochondria, dystrophin, etc. (*Therapeutics*, 30: 1302-1306 (1996)).

The method for treating a patient of the above disease includes a method comprising acquiring the cells having the potential to differentiate into cardiomyocytes of the present invention from a patient of the disease, introducing the wild type gene corresponding to the causative gene of the disease into the cells, and transplanting the cells to the patient's heart. The normal gene is inserted into the vector for gene therapy described in the above 6(1), and then can be introduced into the cells having the potential to differentiate into cardiomyocytes of the present invention using the vector for gene therapy described in the above 6(1).

8. Methods for obtaining antibody which specifically recognizes surface antigen specific for cells having the potential to differentiate into cardiomyocytes

Methods for preparing antibodies which specifically recognize surface antigens expressed in the cells having the potential to differentiate into cardiomyocytes of the present invention are described below.

The antibodies which recognize the surface antigens expressed specifically in the cells having the potential to differentiate into cardiomyocytes of the present invention are useful in the purity test and purification of the cells required for applying the cells to the therapy of heart diseases such as myocardial infarction.

In order to obtain the antibody, an antigen is administered subcutaneously, intravenously or intraperitoneally to a non-human mammal, such as rabbit or goat, or 3 to 20-weeks-old rat, mouse or hamster together with an appropriate adjuvant, such as complete Freund's adjuvant, aluminum hydroxide gel or pertussis vaccine. As the antigen, the cells having the potential to differentiate into cardiomyocytes of the present invention (3×10^5 to 5×10^5 cells/animal) or the cell membrane fraction prepared from the cells (1-10 mg/animal) is used.

Administration of the antigen is repeated 3 to 10 times after the first administration at intervals of 1 to 2 weeks. On the 3rd to 7th day after each administration, a blood sample is collected from fundus oculi veniplex and the obtained serum is examined from reactivity to the antigen used for immunization according to enzyme

immunoassay (*Enzyme-Linked Immuno Adsorbent Assay (ELISA)*, Igaku Shoin (1976), *Antibodies - A Laboratory Manual*, Cold Spring Harbor Laboratory (1988)). A non-human mammal whose serum shows a sufficient antibody titer against the antigen used for immunization is employed as a source of serum or antibody-producing cell.

The polyclonal antibody can be prepared by separation and purification from the serum.

For the preparation of the monoclonal antibody, the antibody-producing cell and a myeloma cell derived from a non-human mammal are fused to obtain hybridoma, and the hybridoma is cultured or administered to an animal to cause ascites tumor. The monoclonal antibody can be prepared by separation and purification from the resulting culture or ascites.

Examples of the antibody-producing cells include spleen cells and antibody-producing cells in lymph nodes or peripheral blood, and among these, spleen cells are preferably used.

As the myeloma cells, mouse-derived cell lines are preferably used. Examples of suitable cell lines are P3-X63Ag8-U1 (P3-U1) cell line (*Current Topics in Microbiology and Immunology*, 18: 1 (1978)), which is 8-azaguanine-resistant mouse (BALB/c-derived) myeloma cell line, P3-NS1/1-Ag41(NS-1) line (*European J. Immunology*, 6: 511 (1976)), SP2/0-Ag14(SP-2) line (*Nature*, 276: 269 (1978)),

P3-X63-Ag8653(653) line (*J. Immunology*, 123: 1548 (1979)) and P3-X63-Ag8(X63) line (*Nature*, 256: 495 (1975)).

The hybridoma can be prepared in the following manner.

The antibody-producing cells and the myeloma cells are mixed and suspended in HAT medium (a medium prepared by adding hypoxanthine, thymidine and aminopterin to a normal medium), followed by culturing for 7-14 days. After the culturing, a portion of the culture supernatant is subjected to enzyme immunoassay to select cells which react with the antigen and do not react with the protein containing no antigen. Then, cloning is carried out by limiting dilution method, and cells showing a high and stable antibody titer according to enzyme immunoassay are selected as the monoclonal antibody-forming hybridomas.

Separation and purification of the polyclonal antibodies and the monoclonal antibodies can be carried out using means such as centrifugation, ammonium sulfate precipitation, caprylic acid precipitation, and chromatography using DEAE-Sepharose column, anion exchange column, protein A- or G-column or gel filtration column, alone or in combination.

Sampling cells can be easily tested for expression of the surface antigen expressed in the cells having the potential to differentiate into cardiomyocytes by comparing the reactivity of the thus obtained antibody specifically

which recognizes the surface antigen to the test cells with that to control cells such as hematopoietic stem cells and neural stem cells.

9. Methods for obtaining surface antigen expressed in cells having the potential to differentiate into cardiomyocytes and gene encoding the surface antigen

The genes encoding the surface antigens expressed specifically in the cells having the potential to differentiate into cardiomyocytes can be obtained by the cDNA subtraction method (*Proc. Natl. Acad. Sci. USA*, 85: 5738-5742 (1988)) and the representational difference analysis (*Nucleic Acids Research*, 22: 5640-5648 (1994)), which are methods for obtaining genes showing different expression profiles between two samples of different origins.

First, a cDNA library prepared from the cells having the potential to differentiate into cardiomyocytes is subjected to subtraction using mRNA obtained from control cells other than cells having the potential to differentiate into cardiomyocytes, e.g., hematopoietic stem cells and neural stem cells. Then a subtracted cDNA library with a high content of a gene specifically expressed in the cells having the potential to differentiate into cardiomyocytes is prepared, followed by nucleotide sequence analysis of inserted cDNA in the

subtracted cDNA library from the 5' terminal side randomly to select those having the secretion signal sequence (random sequence analysis). The full length nucleotide sequences of the thus obtained cDNAs are determined to distinguish the proteins encoded by the cDNAs into secretory proteins and membrane proteins.

In the above process, the signal sequence trap method can be used instead of the random sequence analysis (*Science*, 261: 600-603 (1993), *Nature Biotechnology*, 17: 487-490 (1999)). The signal sequence trap method is a method for selectively screening for genes having the secretion signal sequence.

In order to efficiently obtain the specific surface antigens, it is preferred to prepare a signal sequence trap library from the cells having the potential to differentiate into cardiomyocytes using a vector suitable for subtraction and to subject the signal sequence trap library to subtraction using mRNA obtained from control cells such as hematopoietic stem cells and neural stem cells. The thus obtained DNA fragments containing the secretion signal sequence can be used as probes for cloning the full length cDNAs.

The proteins encoded by the cDNAs can be distinguished into secretory proteins and membrane proteins by determining the full length nucleotide sequences of the full length cDNAs.

When the obtained clone DNA, whether it is obtained by the random sequence analysis or the signal sequence trap method, codes for a membrane protein, the specific antibody can be obtained by the above method using the synthetic peptide prepared based on the amino acid sequence presumed from the nucleotide sequence as an antigen.

The membrane proteins encoded by the clones include receptors, which may act on the regulation of specific growth of cells having the potential to differentiate into cardiomyocytes or their differentiation into cardiomyocytes. The clone encoding such a receptor can be used in the search for a ligand of the receptor. When the clone codes for a secretion protein, it can be used directly for the growth or differentiation of the cells having the potential to differentiate into cardiomyocytes.

10. Methods for screening for growth factor for cells having the potential to differentiate into cardiomyocytes and factor inducing the differentiation into cardiomyocytes

Screening for a growth factor for the cells having the potential to differentiate into cardiomyocytes and a factor inducing their differentiation into cardiomyocytes can be carried out by culturing the cells having the potential to differentiate into cardiomyocytes in a serum-free medium in the presence of a test substance and

evaluating the growth or the cardiomyogenic differentiation of the cells.

This screening method is applicable to a wide variety of test substances, for example, secretion proteins such as various cytokines and growth factors, membrane-bound proteins such as cell adhesion molecules, tissue extracts, synthetic peptides, synthetic compounds, and culture broths of microorganisms.

The growth capability can be evaluated by examining the colony forming activity, the BrdU uptake, etc.

The colony forming activity can be examined by scattering the cells having the potential to differentiate into cardiomyocytes of the present invention at a low density.

The BrdU uptake can be examined by immunostaining using an antibody which specifically recognizes BrdU.

The cardiomyogenic differentiation can be evaluated according to a method using spontaneous beating as an indicator, a method using the expression of a reporter gene introduced into the cells as an indicator, and the like.

The method using the expression of a reporter gene introduced into the cells as an indicator is a method in which a vector DNA comprising the promoter of a gene expressed specifically in cardiomyocytes and a reporter gene is introduced into cells having the potential to

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differentiate into cardiomyocytes and the expression of the reporter gene as an indicator is examined using the cells.

The reporter gene includes genes encoding GFP (green fluorescent protein), luciferase or β -galactosidase, and the like.

The promoter of a gene expressed specifically in cardiomyocytes includes cardiac troponin I (cTNI) (*J. Biological Chemistry*, 273: 25371-25380 (1998)).

11. Methods for immortalizing bone marrow cells having the potential to differentiate into cardiomyocytes

When the therapeutic agent according to the present invention is administered to cardiac patients, especially aged patients, it is preferred that the proliferative activity of the cells having the potential to differentiate into cardiomyocytes of the present invention should be potentiated without generating cancer.

The proliferative activity of the cells having the potential to differentiate into cardiomyocytes can be increased without cancer generation by expressing telomerase in the cells.

The methods for expressing telomerase in the cells having the potential to differentiate into cardiomyocytes of the present invention include: a method which comprises inserting TERT gene which is the catalytic subunit of telomerase, specifically, the DNA represented by SEQ ID

NO:32 into a retrovirus vector and introducing the resulting vector into the cells having the potential to differentiate into cardiomyocytes; a method which comprises administering a factor inducing the expression of the TERT gene inherent in the cells having the potential to differentiate into cardiomyocytes to the cells having the potential to differentiate into cardiomyocytes; and a method which comprises introducing a vector containing DNA encoding a factor inducing the expression of the TERT gene into the cells having the potential to differentiate into cardiomyocytes.

The above-described factors inducing the expression of the TERT gene can be selected by introducing a vector DNA to which a reporter gene such as GFP (green fluorescent protein), luciferase, β -galactosidase or the like has been inserted, into the cells having the potential to differentiate into cardiomyocytes.

12. Method of separating cells having the potential to differentiate into cardiomyocytes using antibody

The method for obtaining cells in which a target surface antigen is expressed from extirpated various *in vivo* tissues includes a method using a flow cytometer having a sorting function and a method using magnetic beads.

The sorting function of a flow cytometer can be performed by the droplet charge system, the cell capture

system, etc. (*Perfect Command of Flow Cytometer*, p.14-23, Shujunsha, 1999). In using each of these systems, the expression amount of an antigen can be quantitated by converting the fluorescent intensity emitted from an antibody binding to a molecule expressed on the cell surface into an electric signal. When plural fluorescences are used in combination, the cells can be separated using plural surface antigens. Examples of the fluorescence include FITC (fluorescein isothiocyanate), PE (phycoerythrin), APC (Allo-phycoerythrin), TR (TexasRed), Cy3, CyChrome, Red613, Red670, PerCP, TRI-Color, QuantumRed, etc. (*Perfect Command of Flow Cytometer*, p.3-13, Shujunsha, 1999).

The staining method includes a method in which cells are centrifugally separated from extirpated various *in vivo* tissues such as bone marrow or umbilical blood, and the cells are stained directly with antibodies, and a method in which the cells are once cultured and proliferated in an appropriate medium and then stained with antibodies.

For staining, the target cells are first mixed with a primary antibody, which recognizes a surface antigen, and incubated on ice for 30 minutes to 1 hour. When the primary antibody is labeled with a fluorescence, the cells are washed and then separated with a flow cytometer. When the primary antibody is not labeled with a fluorescence,

the cells are washed and then a secondary antibody labeled with a fluorescence having an activity of binding to the primary antibody is mixed with the cells having reacted with the primary antibody and incubated on ice again for 30 minutes to 1 hour. After washing, the cells stained with the primary and secondary antibodies are separated with a flow cytometer.

By the method using magnetic beads, cells expressing specific target surface antigen can be separated in a large amount. Although this method is inferior in the separation purity to the flow cytometer method as described above, repeated purification ensures a sufficiently high cell purity.

After staining the cells with the primary antibody, the residual primary antibody is eliminated. Then the cells are stained with the secondary antibody bonded to the magnetic beads capable of binding to the primary antibody. After washing away the residual secondary antibody, the cells can be separated using a stand provided with a magnet. The materials and apparatus required in these operations are available from Dynal Biotech.

The magnetic bead method is also usable in eliminating unnecessary cells from cell samples. The StemSep method marketed from Stem Cell Technologies Inc. (Vancouver, Canada) can be used to eliminate these unnecessary cells more efficiently.

Examples of the antibodies to be used in the above-described methods include the antibodies acquired in the above 8, antibodies which recognize hematopoietic cell surface antigens, CD34, CD117, CD14, CD45, CD90, Sca-1, Ly6c or Ly6g, antibodies which recognize vascular endothelial cell surface antigens, Flk-1, CD31, CD105 or CD144, an antibody which recognizes a mesenchymal cell surface antigen, CD140, antibodies which recognize integrin surface antigens, CD49b, CD49d, CD29 or CD41, and antibodies which recognize matrix receptors, CD54, CD102, CD106 or CD44. When these antibodies are used in combination, the target cells can be obtained at a higher purity.

Specifically, in order to obtain CD34-negative, CD117-positive, CD144-negative and CD140-positive cells, CD34-positive cells and CD144-positive cells are eliminated from human bone marrow cells by, for example, the above-described immune magnetic bead method and then a CD117-positive and CD140-positive cell fraction is recovered to separate the target cells.

13. Separation of cardiomyocyte precursor cells using myocardium-specific gene promoter reporter vector

In order to efficiently separate cardiomyocytes or cardiomyocyte precursor cells derived from cells having the potential to differentiate into cardiomyocytes, green

fluorescent protein (GFP) of luminous *Aequorea* can be used as a reporter gene for gene transfer.

Specifically, a vector is constructed by ligating the GFP gene to the downstream of a promoter of a gene specifically expressed in myocardium or a gene specifically expressed in the cells having the potential to differentiate into cardiomyocytes obtained in the above 9. Then, the vector is introduced into the cells having the potential to differentiate into cardiomyocytes. The cells introducing the reporter vector are separated depending on, for example, tolerance to antibiotics followed by the induction of cardiomyogenic differentiation. The differentiation-induced cells exhibit the expression of GFP and emit fluorescence. The cardiomyocytes and cardiomyocyte precursor cells emitting the fluorescence can be easily separated using a flow cytometer (*Perfect Command of Flow Cytometer*, p.44-52, Shujunsha, 1999).

Examples of the promoter of the gene specifically expressed in myocardium include MLC2v and troponin I.

Examples of the vector include the above-described plasmid vectors for animal cells, and adenovirus vectors.

14. Induction of differentiation of cells having the potential to differentiate into cardiomyocytes into various cells

(1) Induction of differentiation of cells having the potential to differentiate into cardiomyocytes into adipocytes

Examples of the method for inducing the differentiation of the cells having the potential to differentiate into cardiomyocytes into adipocytes include a method wherein an activator of a nuclear receptor, PPAR γ , is added to the medium to give a final concentration of 0.4 to 2 μ M. The activator of a nuclear receptor, PPAR γ , includes compounds having a thiazolidione skeleton such as troglitazone, pioglitazone, rosiglitazone and the like.

The examples also include a method wherein the cells are cultured in a medium to which dexamethasone, methyl-isobutylxanthine, insulin and indomethacin have been added to a culture of cells confluent grown over a culture dish to give final concentrations of 1 μ M, 0.5 mM, 0.01 mg/ml and 0.2 mM, respectively.

(2) Induction of differentiation of cells having the potential to differentiate into cardiomyocytes into chondrocytes

Examples of the method for inducing the differentiation of the cells having the potential to

differentiate into cardiomyocytes into chondrocytes include a method wherein aggregates obtained by centrifuging 1×10^5 to 3×10^5 cells are cultured in a medium containing TGF β 3 in a final concentration of 0.01 μ g/ml.

(3) Induction of differentiation of cells having the potential to differentiate into cardiomyocytes into osteoblasts

Examples of the method for inducing the differentiation of the cells having the potential to differentiate into cardiomyocytes into osteoblasts include a method wherein the cells are cultured in a medium containing dexamethasone, ascorbic acid-2-phosphate and β -glycerophosphate in final concentrations of 0.1 μ M, 0.05 mM and 10 mM, respectively.

15. Purification of stem cell using Hoechst 33342

Hoechst 33342 is a DNA binding reagents which can stain viable cells. Since the majority of bone marrow cells are vigorously divided, they are stained markedly lightly but immature cells are stained darkly. It is known that this phenomenon becomes significant in cells having immature ability to exclude pigment by ABC (ATP binding cassette) transporter (H. Nakauchi, *Protein, Nucleic Acid and Enzyme*, 45: 13, 2056-2062 (2000)).

Cells which are stained darkly with Hoechst 33342

can be separated from the bone marrow by staining bone marrow cells with Hoechst 33342 and then analyzing them by carrying out double staining of a short wavelength and a long wavelength by applying UV laser using FACS. Immature cells which do not incorporate Hoechst 33342 can be fractionated as side population (Goodell, M.A. et al., *J. Exp. Med.*, 183: 1797-1806 (1996), http://www.bcm.tmc.edu/genetherapy/goodell/new_site/index2.html).

BRIEF EXPLANATION OF THE DRAWINGS

Fig. 1 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using a biotinylated anti-mouse CD105 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 2 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using a biotinylated anti-mouse Flk1 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 3 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-

mouse CD31 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 4 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using a biotinylated anti-mouse CD144 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 5 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD34 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 6 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD117(c-kit) antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody

reaction, and the solid line is a result of a negative control.

Fig. 7 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD14 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 8 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD45 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 9 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD90 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 10 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse Ly6A/E(Sca-1) antibody which was measured by a flow

cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 11 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse Ly6c antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 12 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse Ly6g antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 13 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using a biotinylated anti-mouse CD140 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 14 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD49b antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 15 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD49d antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 16 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD29 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 17 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD54 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out

with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 18 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD102 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 19 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD106 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

Fig. 20 shows a result of the antibody reaction of KUM2 cell (A) or BMSC cell (B) using an FITC-labeled anti-mouse CD44 antibody which was measured by a flow cytometer. The ordinate and abscissa show the number of cells and the fluorescence intensity, respectively. The area painted out with gray is a result of the antibody reaction, and the solid line is a result of a negative control.

The present invention are illustrated below based on the following examples in more detail.

BEST MODE FOR CARRYING OUT THE INVENTION

Example 1

Isolation and culture of bone marrow cells having the potential to differentiate into cardiomyocytes from mouse bone marrow:

Ten 5-weeks-old C3H/He mice were anesthetized with ether and sacrificed by cervical dislocation. Each mouse was laid in half-lateral position and sufficiently disinfected with 70% ethanol. The skin around the femur was widely opened and the quadriceps femoris covering the femur was excised with scissors. The femur was put out of the knee joint with scissors and the muscle on the back side of the femur was removed. Then, the femur was put out of the hip joint with scissors and taken out. After the muscle on the femur was removed with scissors to expose the whole femur, the femur was cut at both ends using scissors. A needle (23G, TERUMO) was attached to a 2.5 ml syringe and about 1.5 ml of IMDM containing 20% FCS was put into the syringe. The needle of the syringe was put into the femur from the cut end of the knee joint side and the culture medium was injected into the bone marrow, whereby bone marrow cells were pressed out of the bone into a test tube. The thus obtained cell were cultured in IMDM supplemented with 20% FCS, 100 mg/ml penicillin, 250 ng/ml streptomycin and 85 mg/ml amphotericin at 33°C using a 5% CO₂-incubator.

As a result of a series of passages, the cells were homogenized into mesenchymal cells and hematopoietic cells disappeared.

After culturing for about 4 months under the above conditions, immortalized cells were selected and diluted to establish 192 cell lines respectively derived from single cells (hereinafter referred to as bone marrow-derived first passage immortalized cell lines). To each of these clone-derived cell lines was added 5-aza-C at a final concentration of 3 μ M, and the cells were cultured for 24 hours. After culturing for further 2 weeks in IMDM, clones that produced spontaneously beating cells were selected. Among the bone marrow-derived first passage immortalized cell lines (192 cell lines), three cell lines were found to have the potential to differentiate into cardiomyocytes. One of three cell lines is KUM2. Hereinafter, unless otherwise indicated, the bone marrow cell KUM2 and mouse bone marrow-derived pluripotent stem cells (BMSC) described below were cultured in IMDM supplemented with 20% FCS, 100 mg/ml penicillin, 250 ng/ml streptomycin and 85 mg/ml amphotericin at 33°C using a 5% CO₂-incubator. When the KUM2 cells were exposed to 5-aza-C having a final concentration of 3 μ M for 24 hours, nonspecific differentiation into spontaneously beating cardiomyocytes was induced. However, the frequency is very low (one or less per 10⁷ cells).

However, cells surrounding the spontaneously beating cells derived from the KUM2 cells were collected using a cloning syringe to observe at least two cells of the mouse bone marrow-derived pluripotent stem cells (BMSC) having a high proliferation potentiality (FERM BP-7043) and cells differentiated into cardiomyocytes by proliferation under limited times (hereinafter referred to as "cardiomyocyte precursor cells"). BMSC cells isolated by cloning syringe was cloned by selecting immortalized cells in the course of multiple passage. It was observed that the differentiation of the BMSC cells was induced at least 100 times as efficient as the parent cell line, KUM2. And to the cardiomyocyte precursor cells, 5-aza-C was added, followed by culturing for 24 hours, and further culturing in IMDM for 2-3 weeks, so that a larger number of spontaneously beating cells were efficiently obtained. The cardiomyocyte precursor cells showed mononuclear fibroblast-like morphology under the proliferation conditions and expression of myocardial contractile proteins was hardly observed. However, induction of final differentiation with 5-aza-C caused a remarkable change in the morphology of the cells.

About one week after the induction of differentiation, parts of cells showed enlargement of cytoplasm and showed a ball-like or stick-like appearance. Such cells began spontaneously beating afterwards but

spontaneous beating was still rare at this stage. Two weeks after the induction of differentiation, the cells began spontaneously beating. The spontaneously beating cells connected lengthwise with one another to form myotube-like structures. Three weeks after the induction of differentiation, many cells were connected in a column and simultaneously contracted. Four weeks after the induction of differentiation, all of the directly connected cells on the culture dish showed simultaneous contraction and formed a myocardial tissue-like structure. The heart of a mouse contracts at a heart rate of 300-400 per minute. On the other hand, the cardiomyocytes differentiated from the cells derived from mouse adult bone marrow showed regular contraction at a rate of 120-250 per minute under the culture conditions.

Example 2

Characteristics of the Cardiomyocytes Derived from Mouse Bone Marrow Cells:

The spontaneously beating cardiomyocyte-like cells produced from the bone marrow cells were examined for the characteristics of cardiomyocytes.

Total RNAs were obtained from the bone marrow-derived first passage immortalized cell line, the mouse bone marrow-derived pluripotent stem cells (BMSC), and the cardiomyocytes derived from the cardiomyocyte precursor

cells, which were obtained in Example 1, using Trizol Reagents (manufactured by GIBCO BRL). Then, first strand cDNAs were synthesized from the total RNAs as the substrates using SuperscriptII reverse transcriptase (manufactured by GIBCO BRL).

In order to examine the expression of cardiomyocyte-specific genes, quantitative PCR was carried out using the first strand cDNAs as the substrates and using the synthetic DNAs having the nucleotide sequences represented by SEQ ID NOS:33 to 58. As the cardiomyocyte-specific genes, ANP and BNP, which are natriuretic peptides, α -MHC and β -MHC, which are myosin heavy chains, α -skeletal actin and β -skeletal actin, which are actins, MLC-2a and MLC-2v, which are myosin light chains, and Nkx2.5/Csx, GATA4, TEF-1, MEF-2C, MEF-2D and MEF-2A, which are cardiomyocyte-specific transcription factors, were employed.

For the amplification of the above genes, the synthetic DNAs having the nucleotide sequences shown in the following SEQ ID NOS were respectively used: ANP, SEQ ID NOS:33 and 34; BNP, SEQ ID NOS:35 and 36; α -MHC, SEQ ID NOS:37 and 38; β -MHC, SEQ ID NOS:39 and 40; α -skeletal actin, SEQ ID NOS:41 and 42; β -skeletal actin, SEQ ID NOS:43 and 44; MLC-2a, SEQ ID NOS:45 and 46; MLC-2v, SEQ ID NOS:47 and 48; Nkx2.5/Csx, SEQ ID NOS:49 and 50; GATA4, SEQ ID NOS:51 and 52; TEF-1, SEQ ID NOS:53 and 54; MEF-2C, SEQ

ID NOS:55 and 56; MEF-2D, SEQ ID NOS:57 and 58; and MEF-2A, SEQ ID NOS:59 and 60.

In cardiomyocytes produced by induced differentiation in vivo, myocardial contractile proteins have different isoforms according to the difference in stage, i.e., fetal period, new-born period or maturation period, or the difference in type, i.e., atrial or ventricular, so that the rate and energy efficiency of myocardial contraction may vary appropriately.

In the case of the bone marrow cells which differentiate into cardiomyocytes in vitro, α -skeletal actin was expressed at higher levels than α -cardiac actin in the expression pattern of isoforms; β -MHC was expressed at higher levels than α -MHC in the myosin heavy chain; and MLC-2v was expressed, whereas MLC-2a expression was not observed in the myosin light chain.

After the induction of differentiation of the bone marrow cells into cardiomyocytes in vitro, the expression of the natriuretic peptides, ANP and BNP, was observed. In view of the above expression pattern of myocardial contractile proteins, it is considered that the bone marrow cells which differentiated into cardiomyocytes in vitro have a phenotype specific to fetal ventricular cardiomyocytes.

In the bone marrow cells which differentiated into cardiomyocytes in vitro, the expression of genes coding for

Nkx2.5/Csx, GATA4, MEF-2A, MEF-2C, MEF-2D or TEF-1 was observed. The genes coding for these transcription factors were not expressed in the bone marrow-derived first passage immortalized cell lines during proliferation. In the bone marrow-derived cardiomyocyte precursor cells during proliferation, the expression of genes coding for Nkx2.5/Csx, GATA4 or MEF-2C was observed. The expression of MEF-2A and MEF-2D was induced later with the induction of cardiomyogenic differentiation.

The action potentials of the bone marrow cells which differentiated into cardiomyocytes *in vitro* were recorded using glass microelectrodes. The cells were cultured in IMDM supplemented with 1.49 mM CaCl₂, 4.23 mM KCl and 25 mM HEPES (pH 7.4), and the action potentials of the cells were measured at 25°C under an inverted phase-contrast optic (Diaphoto-300, manufactured by Nikon). The glass microelectrodes were filled with 3M KCl and the electrode resistance was set at 15-30 Ω in the glass microelectrodes. The membrane potentials were measured with current clamp mode using MEZ-8300 (manufactured by Nihon Kohden). The data were recorded on thermal recording papers using RTA-1100M (manufactured by Nihon Kohden). As a result, it was found that the bone marrow cells which differentiated into cardiomyocytes *in vitro* were classified into two types of action potentials: one is sinus node-like action potential and the other is ventricular myocyte-like

action potential. These two type cells of action potentials had the following characteristics in common: (1) a long action potential duration, (2) a relatively shallow resting potential, (3) pacemaker-like slow depolarization of resting potential. The ventricular myocyte-like action potential showed the peak- and dome-like pattern having the phase 1 action potential. The sinus node-like action potential showed the action potential duration, diastolic membrane potential and action potential amplitude which are similar to those previously reported with the action potentials of sinus node cells of rabbits and rats. In comparison, the ventricular myocyte-like action potential had a tendency to show a deep resting membrane potential and a high action potential amplitude. During the 2-3 weeks after the induction of differentiation, the sinus node-like action potential was recorded for all the cells. The ventricular myocyte-like action potential was first recorded about 4 weeks after the induction of differentiation and its incidence gradually increased with the passage of time.

Example 3

Stimulation of cardiomyogenic differentiation using cytokine:

The following experiment was conducted to investigate the stimulating effect of cytokines on the

cardiomyogenic differentiation of the mouse bone marrow cells having the potential to differentiate into cardiomyocytes induced by 5-aza-C.

The mouse bone marrow-derived pluripotent stem cells (BMSC) having the potential to differentiate into cardiomyocytes were plated into 60-mm culture dishes and 60 mm fibronectin-coated dishes (Becton Dickinson) at a density of 2×10^4 cells/ml and cultured at 33°C in a 5% CO₂-incubator.

On the next day, 5-aza-C was added to each culture medium in a final concentration of 3 μ M, followed by culturing with the following 3 different treatments, with addition of PDGF (culture dish A), both PDGF and retinoic acid (culture dish B) or without addition of any compound (culture dish C) (final concentration: PDGF, 10 ng/ml; retinoic acid, 10^{-9} M).

On the next day, the medium was replaced with a fresh medium to remove 5-aza-C therefrom. Then, PDGF was added to the culture dish A until the final concentration of PDGF came to be 10 ng/ml, while PDGF and retinoic acid were added to the culture dish B until the final concentrations of PDGF and retinoic acid came to be 10 ng/ml and 10^{-9} M, respectively. Two and four days thereafter, the medium was replaced and the PDGF or retinoic acid was further added.

Four weeks after the addition of the chemicals, the cell morphology was observed with a phase-contrast microscope. As a result, about 30% of the cells in the culture dish containing 5-aza-C alone differentiated into myotubes, while about 40% of the cells in the culture dish containing PDGF and about 50% of the cells in the culture dish containing PDGF together with retinoic acid differentiated into myotubes. In the three groups of the fibronectin-coated dishes, the ratio of the cells differentiated into myotubes was about 10% higher than in the three groups of the culture dishes.

RNAs were collected from the myotubes thus obtained. And genes expressed in the myotubes were analyzed with quantitative PCR analysis using the synthetic oligonucleotides represented by SEQ ID NOS:71 to 78. As a result, PDGF or retinoic acid promoted the expression of MyoD and fTnI genes relating to a skeletal muscle but not cTnI or ANP specifically relating to a myocardium.

Next, mouse bone marrow-derived pluripotent stem cells (BMSC) having the potential to differentiate into cardiomyocytes were inoculated in a 60-mm culture dish at a density of 2×10^4 cells/ml and cultured using an incubator at 33°C under 5% of CO₂.

On the next day, 5-aza-C was added to the liquid culture medium to give a final concentration of 3 μ M. Furthermore, five treatments differing from each other were

performed by adding FGF-8 to give a final concentration of 10 ng/ml (culture dish D); adding ET-1 to give a final concentration of 10 ng/ml (culture dish E); adding a midkine to give a final concentration of 10 ng/ml (Culture dish F); adding BMP4 to give a final concentration of 10 ng/ml (culture dish G); and adding no compound (culture dish H), followed by culturing.

On the next day, the medium was replaced by a fresh medium to eliminate 5-aza-C therefrom. Then, FGF-8 was added to the culture dish D to give a final concentration of 10 ng/ml; ET-1 was added to the culture dish E to give a final concentration of 10 ng/ml; the midkine was added to the culture dish F to give a final concentration of 10 ng/ml; and BMP4 was added to the culture dish G to give a final concentration of 10 ng/ml, followed by culturing. Two and four days thereafter, the medium was replaced and the FGF-8, ET-1, midkine or BMP4 was further added.

Four weeks after the addition of 5-aza-C, the cell morphology was observed with a phase-contrast microscope. As a result, about 30% of the cells in the culture dish containing 5-aza-C alone differentiated into myotubes, while about 50% of the cells in the culture dishes containing FGF-8, ET-1, midkine or BMP4 differentiated into myotubes respectively.

RNAs were collected from the myotubes thus obtained. And genes expressed in the myotubes were analyzed with

quantitative PCR using the synthetic oligonucleotides represented by SEQ ID NOS:71 to 78. As a result, the FGF-8, ET-1, midkine and BMP4 each individually promoted the expression of cTnI and ANP gene which are myocardium-specific genes.

Example 4

Induction of differentiation of bone marrow-derived stem cells into cardiomyocytes using DMSO:

According to the method described in Example 1, mouse bone marrow-derived pluripotent stem cells (BMSC) having the potential to differentiate into cardiomyocytes were obtained and cultured for 24 hours in the presence of 10 μ M DMSO instead of 3 μ M 5-aza-C. The medium was replaced with IMDM, followed by culturing for 6 weeks.

As a result, the stem cells were induced to differentiate into beating cardiomyocytes. The produced cells expressed Nkx2.5/Csx and GATA4 genes and were found to be cardiomyocytes having the same properties as those obtained by the 5-aza-C treatment. This result indicates that cardiomyogenic differentiation requires demethylation of chromosomal DNA, which is a function common to 5-aza-C and DMSO.

Example 5

Demonstration that mouse bone marrow-derived pluripotent cells having the potential to differentiate into cardiomyocytes are pluripotent stem cells and cardiomyocyte precursor cells:

It was demonstrated above that the beating cells differentiated from the mouse bone marrow-derived pluripotent stem cell (BMSC) have the properties of cardiomyocytes. In this example, a single cell marking experiment was carried out to examine whether cardiomyocyte precursor cells are present in the mouse bone marrow-derived pluripotent stem cells (BMSC) having the potential to differentiate into cardiomyocytes, or whether more undifferentiated stem cells which can differentiate into not only cardiomyocytes, but also, for example, adipocytes and other cell types are present.

Specifically, a GFP gene was inserted into a virus vector and the vector was transfected into a cell for labeling prior to induction of differentiation, and the labeled cell was induced to differentiate to observe what kind of cell is produced by differentiation.

First, retrovirus vector plasmid GAR3-GFP which expresses the GFP gene products and plasmid vector pCMV-Eco which expresses the Ecotropic gene products were treated according to the alkali neutralization method and the PEG

precipitation method described in *Molecular Cloning, A Laboratory Manual*, 2nd ed. to obtain DNAs of high purity.

One day before DNA transfection, 293 cells carrying the gag and pol genes which had reached confluence were passaged into a 10-cm dish by 1/5 dilution and cultured overnight at 37°C in a 5% CO₂-incubator.

Transfection was carried out as follows.

GAR3-GFP retrovirus vector plasmid DNA (15 µg) and pCMV-Eco plasmid vector DNA (5 µg) were dissolved in 0.5 ml of 250 mM CaCl₂ (pH 6.95). The resulting solution was added dropwise to a 15 ml tube containing 0.5 ml of 2× BBS (50 mM BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), 280 mM NaCl and 1.5 mM Na₂HPO₄ (pH 6.95)) and the tube was allowed to stand at room temperature for 10 minutes. The resulting DNA solution was added dropwise to the 293 cell culture prepared on the preceding day, followed by culturing at 37°C in a 5% CO₂-incubator. On the next day, the medium was replaced with a fresh medium, followed by culturing at 37°C in the 5% CO₂-incubator.

Two days after the medium replacement, the culture supernatant was filtered through a 0.45 µm filter (manufactured by Millipore) to recover a solution containing the virus vector. The obtained solution was diluted to 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ with IMDM.

The mouse bone marrow-derived pluripotent stem cells having the potential to differentiate into

cardiomyocytes into which the virus vector was to be introduced were plated into 6-well dishes at a density of 2×10^4 cells/well on the day before virus infection.

To the diluted virus vector solution, hexadimethine bromide (polybrene) (manufactured by Sigma) was added to give a final concentration of 8 $\mu\text{g/ml}$. After 2 ml of the culture supernatant of the mouse bone marrow-derived pluripotent stem cells (BMSC) having the potential to differentiate into cardiomyocytes was replaced with 2 ml of the virus solution, culturing was carried out at 33°C in a 5% CO_2 -incubator. Five hours later, the culture supernatant was replaced with a fresh IMDM, followed by culturing at 33°C in the 5% CO_2 -incubator.

After culturing for 2 days, the cells were observed for GFP expression by a fluorescence microscope to obtain cell populations containing one GFP-positive cell in 1000 cells.

The obtained cells were plated into 35 mm glass base dishes (manufactured by Asahi Techno Glass) at a density of 8×10^3 cells/dish followed by culturing at 33°C in a 5% CO_2 -incubator.

On the next day, 5-aza-C (manufactured by Sigma), PDGF-BB (manufactured by Peprotech) and all trans retinoic acid (manufactured by Sigma) were added to the dishes to give final concentrations of 3 μM , 10 ng/ml and 10^{-9} M, respectively. Two days and four days after the addition,

the medium was replaced with a fresh medium and PDGF-BB (hereinafter referred to as "PDGF") and all trans retinoic acid were added at the same concentrations as above.

Four weeks after, the cultures were observed under a fluorescence microscope to examine the mode of differentiation of the GFP-positive cells. As a result, the following three kinds of cell populations were observed; cell populations in which all the GFP-positive cells were cardiomyocytes; cell populations in which cardiomyocytes and undifferentiated stem cells were GFP-positive; and cell populations in which cardiomyocytes, adipocytes and undifferentiated stem cells were GFP-positive. It has thus been found that differentiation is stochastically derived from pluripotent stem cells through myocardial stem cells and then cardiomyocyte precursor cells. This result also indicates that the mouse bone marrow cells having the potential to differentiate into cardiomyocytes comprise pluripotent stem cells.

Example 6

Promotion of differentiation into cardiomyocytes by forced expression of transcription factors:

The following experiment was carried out to examine the effect of the forced expression of transcription factors relating to cardiomyogenic differentiation on the cardiomyogenic differentiation of the bone marrow-derived

pluripotent stem cells (BMSC) having the potential to differentiate into mouse cardiomyocytes.

That is, the Nkx2.5/Csx or GATA4 gene was introduced into the cells using a virus vector prior to induction of differentiation, and then the cells were induced to differentiate to examine the efficiency of cardiomyogenic differentiation.

In order to express the Nkx2.5/Csx, Nkx2.5/Csx was inserted into retrovirus vector plasmid pCLNCX (manufactured by Imgenex) to prepare pCLNC-Nkx2.5/Csx.

Furthermore, in order to express GATA4, GATA4 was inserted into plasmid pCLPCX in which the G418-resistant gene portion in retrovirus vector plasmid pCLNCX (manufactured by Imgenex) had been replaced with puromycin-resistant genes, to prepare pCLPC-GATA4. The retrovirus vector plasmids pCLNC-Nkx2.5/Csx and pCLPC-GATA4 and plasmid vector pCMV-Eco (manufactured by Imgenex) which expresses the Ecotropic gene were treated according to the alkali neutralization method and the PEG precipitation method described in *Molecular Cloning, A Laboratory Manual*, 2nd ed., etc. to obtain DNAs having high purity.

One day before DNA transfection, 293 cells carrying the gag and pol gene which had reached confluence were passaged into a 10-cm dish by 1/5 dilution followed by culturing overnight at 37°C in a 5% CO₂-incubator.

Transfection was carried out as described below.

15 µg of retrovirus vector DNA, pCLNC-Nkx2.5/Csx or pCLPC-GATA4, and 5 µg of plasmid vector, pCMV-Eco, were added and dissolved in 0.5 ml of 250 mM CaCl₂ (pH 6.95). The resulting solution was added dropwise to a 15 ml tube containing 0.5 ml of 2×BBS (50 mM BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), 280 mM NaCl and 1.5 mM Na₂HPO₄ (pH 6.95)) and the tube was allowed to stand at room temperature for 10 minutes. The resulting DNA solution was added dropwise to the 293 cell culture prepared on the preceding day, followed by culturing at 37°C in a 5% CO₂-incubator. On the next day, the medium was replaced with a fresh medium, followed by culturing at 37°C in the 5% CO₂-incubator.

Two days after the medium replacement, the culture supernatant was filtered through a 0.45 µm filter (manufactured by Millipore) to recover a solution containing the virus vector.

The mouse bone marrow-derived pluripotent stem cells (BMSC) having the potential to differentiate into cardiomyocytes into which the virus vector was to be introduced were plated into 6-well dishes at a density of 2×10^4 cells/well on the day before virus infection.

To the obtained virus vector solution, hexadimethrine bromide (polybrene) (manufactured by Sigma) was added to give a final concentration of 8 µg/ml. The culture medium was replaced with the culture medium for the

mouse bone marrow-derived pluripotent stem cells (BMSC) having the potential to differentiate into cardiomyocytes, followed by culturing at 33°C in a 5% CO₂-incubator. Five hours later, the medium was replaced with a fresh IMDM, followed by culturing at 33°C in the 5% CO₂-incubator, and further culturing for 2 days.

G418 was added to the cells infected with the virus produced by transferring pCLNC-Nkx2.5 and pCMV-Eco to give a final concentration of 300 µg/ml, followed by culturing for further 7 days.

Separately, puromycin was added to the cells infected with the virus produced by transferring pCLPC-GATA4 and pCMV-Eco to give a final concentration of 300 ng/ml, followed by culturing for further 7 days.

During this period, both cells partly died and were detached from the dish. The surviving cells were suspended with trypsin followed by plating into new culture dishes.

The obtained stable transformants for expression of Nkx2.5/Csx or GATA4 were induced for differentiation by the method in the above Example 3, and thus the differentiation efficiency into cardiomyocytes was examined.

The NKx2.5 forced expressing bone marrow cells (BMSC-Nkx2.5) having the potential to differentiate into cardiomyocytes and the GATA4 forced expressing bone marrow cells (BMSC-GATA4) having the potential to differentiate into cardiomyocytes were plated into 60-mm culture dishes

at a density of 2×10^4 cells/ml, followed by culturing at 33°C in a 5% CO₂-incubator. On the next day, 5-aza-C was added to each culture medium to give a final concentration of 3 μ M. After continuing the culturing at 33°C in a 5% CO₂-incubator for further 24 hours, the medium was replaced with a fresh medium to eliminate 5-aza-C, followed by culturing for additional 4 weeks. When observed with a phase-contrast microscope, the number of myotube showed no large change caused by the forced expression of Nkx2.5/Csx or GATA4. Next, RNAs were collected from the myotubes thus obtained and genes expressed in the myotubes were analyzed with quantitative PCR using the synthetic oligonucleotides represented by SEQ ID NOS:71 to 78. As a result, it was observed that the forced expression of Nkx2.5/Csx or GATA4 promoted the expression of cTnI and ANP which are myocardium-specific genes.

To simultaneously express both of the Nkx2.5/Csx and GATA4 genes in bone marrow cells having the potential to differentiate into cardiomyocytes, a retrovirus vector plasmid pCLPC-GATA4 was treated as described above and bone marrow cells (BMSC-NKx2.5) with the forced expression of Nkx2.5/Csx having the potential to differentiate into cardiomyocytes were infected with the recombinant virus thus constructed. Next, puromycin was added to give a final concentration of 300 ng/ml to obtain a drug-tolerant clone (BMSC-Nkx2.5-GATA4).

The Nkx2.5/Csx and GATA4 co-forced expressing bone marrow cells (BMSC-Nkx2.5-GATA4) having the potential to differentiate into cardiomyocytes were plated into a 60-mm culture dish at a density of 2×10^4 cells/ml, followed by culturing at 33°C in a 5% CO₂-incubator.

On the next day, 5-aza-C was added to the culture medium to give a final concentration of 3 μ M. After culturing at 33°C in a 5% CO₂-incubator for further 24 hours, the medium was replaced with a fresh medium to eliminate 5-aza-C, followed by culturing for 4 weeks. When observed with a phase-contrast microscope, the number of myotube showed no large change caused by the forced expression of the Nkx2.5/Csx and GATA4 genes. However, the number of beating cardiomyocyte was 50 times or more elevated than bone marrow cells with no forced expression of these genes having the potential to differentiate into cardiomyocytes. Next, RNAs were collected from the myotubes thus obtained and genes expressed in the myotubes were analyzed with quantitative PCR using the synthetic oligonucleotides represented by SEQ ID NOS:71 to 78. As a result, it was observed that the forced expression of Nkx2.5/Csx and GATA4 promoted the expression of cTnI and ANP which are myocardium-specific genes.

Example 7

Promotion of differentiation into cardiomyocytes by combination of the forced expression of transcriptional factors with cytokines:

By combining the above-described transcriptional factors (Nkx2.5/Csx and GATA4) promoting the differentiation into cardiomyocytes with cytokines (FGF-8, ET-1, midkine and BMP4), effects on the differentiation into cardiomyocytes were analyzed.

The Nkx2.5/Csx and GATA4 co-forced expressing bone marrow cells (BMSC-Nkx2.5-GATA4) having the potential to differentiate into cardiomyocytes were plated into a 60-mm culture dish at a density of 2×10^4 cells/ml and cultured at 33°C in a 5% CO₂-incubator.

On the next day, 5-aza-C was added to the culture medium to give a final concentration of 3 µM. Furthermore, 5 treatments differing from each other were carried out by adding FGF-8 to give a final concentration of 10 ng/ml (culture dish I); adding ET-1 to give a final concentration of 10 ng/ml (culture dish J); adding midkine to give a final concentration of 10 ng/ml (culture dish K); adding BMP4 to give a final concentration of 10 ng/ml (culture dish L); and adding nothing (culture dish M), followed by culturing.

On the next day, the medium was replaced with a fresh medium to eliminate 5-aza-C. Then, FGF-8 was added

to the culture dish I to give a final concentration of 10 ng/ml; ET-1 was added to the culture dish J to give a final concentration of 10 ng/ml; midkine was added to the culture dish K to give a final concentration of 10 ng/ml; and BMP4 was added to the culture dish L to give a final concentration of 10 ng/ml, followed by culturing. Two and four days thereafter, furthermore, the medium was replaced, and the FGF-8, ET-1, midkine or BMP4 was added.

Four weeks after the addition of 5-aza-C, the cell morphology was observed with a phase-contrast microscope. As a result, about 30% of the cells in the culture dish containing 5-aza-C alone were converted into myotubes, while about 50% of the cells in the culture dishes containing FGF-8, ET-1, midkine or BMP4 differentiated into myotubes respectively. On the other hand, the addition of FGF-8, ET-1, midkine or BMP4 caused no increase in beating cardiomyocytes.

From the myotubes thus obtained, RNAs were collected and genes expressed in the myotubes were subjected to quantitative PCR analysis using the synthetic oligonucleotides represented by SEQ ID NOS:71 to 78. As a result, the FGF-8, ET-1, midkine and BMP4 did not further promote the expression of cTnI and ANP which had been promoted by the forced expression of Nkx2.5/Csx and GATA4.

Example 8

Transplantation of mouse having the potential to differentiate into cardiomyocytes into heart:

In order to examine whether or not bone marrow cells having the potential to differentiate into cardiomyocytes would differentiate into myocardia and thus take into the heart, the GFP labeled bone marrow cells (BMSC-GFP) having the potential to differentiate into cardiomyocytes as prepared in Example 5 were employed as donor cells for the transplantation into mouse. Specifically, the following procedure was performed. The GFP-labeled BMSCs were transiently treated with 5-aza-C for 24 hours, then suspended in PBS to give a concentration of 1×10^8 cells/ml and stored on ice until immediately before the transplantation. It had been confirmed by 0.05% erythrosine-staining that BMSCs could survive at a ratio of about 95%.

On the other hand, the recipient C3H/He mice (available from Charles River Japan) were anesthetized with ether, and the anesthesia was maintained by intraperitoneally administering 30 mg of thiopental using a Terumo syringe (1 ml) manufactured by Terumo Corp. The legs of each mouse were fixed on a cork board with tape, and its upper jaw was also fixed on the cork board with rubber in such a manner that the neck leaned back. At this stage, electrocardiography electrodes were put into both

upper limbs and right side lower limb to monitor the electrocardiogram. Next, the cervix was incised about 1 cm along the trachea using Mayo scissors (NONAKA RIKAKI CO., LTD, NK-174-14), the thyroid gland was stripped to the right and left sides using a baby cotton swab manufactured by Hakujuji, and then muscles around the trachea were incised using micro scissors (NONAKA RIKAKI CO., LTD, NY-334-08) to expose the trachea. Next, the trachea was incised in about 1 mm width using a micro-feather (a surgical knife), a needle of Surflow Flash (22G) manufactured by Terumo deformed into J-shape was inserted into the opening and taken out from the oral cavity, and then the syringe of Surflow Flash (20G) was inserted into the trachea using the needle as a guide. By connecting a respirator (MODEL SN-480-7, manufactured by SHINANO SEISAKUSHO) to the syringe, 100% oxygen was flowed at a rate of 1 ml/minute to start artificial respiration with a tidal volume of 1 ml and a respiration frequency of 120/minute. Since air leaks out from the guide needle-inserted opening, the skin around the trachea was closed by covering the trachea using mosquito forceps (manufactured by NONAKA RIKAKI CO., LTD.). Next, a region of about 2 cm from the sternal pedicel toward the cervix was incised using Mayo scissors and then the sternum was incised about 2 cm from the sternal pedicel toward the cervix. Bleeding was stopped using a bipolar electric knife, and then a 30G

needle (metal hub exchange needle N730) manufactured by GL Science was connected to the Terumo syringe (1 ml) manufactured by Terumo Corp and 0.1 ml of a solution prepared by suspending the donor cells in PBS was injected into the apical region. Next, the sternum and the skin were closed using 4-0 ETHIBOND X761 manufactured by ETHICON, and the skin of the cervix was closed using the same suture. After confirmation of the turn up of spontaneous respiration, the respirator was taken out, and an infant warmer was heated to 37°C to wait vigilance of the animal therein. Also, the procedure of this test was carried out using DESIGN FOR VISON 4.5x SURGICAL TELESCOPES.

Tissues were taken out from the mouse 77 days after the transplantation, fixed with 10% formalin and embedded in paraffin. The embedded tissues were sliced with a microtome into pieces of 6 µm in thickness and adhered to slide glasses which had been coated with poly-L-lysine. After eliminating paraffin by immersing in 10% xylene, the samples were washed with ethanol and then immersed in 0.3% H₂O₂ for 30 minutes, followed by a pretreatment for the antibody reaction.

Then, the samples were washed with PBS and blocked by reacting with a 5% normal swine serum solution. After blocking, the samples were washed with PBS and then subjected to the antibody reaction by allowing to stand at 4°C overnight together with a mouse anti-GFP monoclonal

antibody (manufactured by CLONTECH). After washing with PBS, the samples were allowed to react with a peroxidase-labeled dextran-bonded goat anti-mouse immunoglobulin antibody (manufactured by DACO) at room temperature for 30 minutes. After washing with PBS again, a coloring solution (10 µg/ml 3,3'-diaminobenzidine (DAB) tetrahydrochloride, 0.01% H₂O₂, 0.05 M Tris-HCl (pH 6.7)) was added and allowed to react for about 10 minutes. Then, the reaction mixture was washed with PBS to stop the reaction. Furthermore, the slide glasses were stained with methyl green. The part of continuous pieces were stained with hematoxylin/eosin to clarify the morphology of the tissue pieces.

As a result, GFP-positive cells were observed in the cardiomyocytes and the vascular endothelial cells.

Thus, it can be concluded that the transplanted mouse bone marrow cells had differentiated into the cardiomyocytes and the vascular endothelial cells.

Example 9

Promotion of differentiation into cardiomyocytes by cultured cardiomyocyte-derived factor:

As shown in Example 8, the bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes were differentiated into the cardiomyocytes when transplanted into the heart. This result suggests that cardiomyocytes *per se* expresses a factor inducing the

differentiation of bone marrow cells into cardiomyocytes. To examine this hypothesis, a mouse fetal heart was taken out from a C3H/He mouse on the day 16 of pregnancy and a primary culture cell line of cardiomyocytes (hereinafter referred to as the "cultured cardiomyocytes") was established in accordance with a publicly known method (*Development of Method for Studying Heart and Blood*, ed. by Setsuro Ebashi, Gakkai Shuppan Senta, (1983)).

To examine whether or not a factor secreted from the cultured cardiomyocytes has an activity of promoting heart differentiation, 5×10^6 cultured cardiomyocytes were cultured in a culture dish for 72 hours. Next, the culture supernatant was filtered through a $0.45 \mu\text{m}$ filter (manufactured by Millipore). The culture supernatant thus filtered was mixed with the equivalent amount of a medium to give a culture medium (hereinafter referred to as the "conditioned medium") containing the factor secreted from the cultured cardiomyocytes.

Bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes or Nkx2.5 and GATA4 forced expressing bone marrow cells (BMSC-Nkx2.5-GATA4) having the potential to differentiation into cardiomyocytes were cultured 6-cm culture dishes at a density of 1×10^5 cells and then the medium was replaced with the conditioned medium. At this point, 5-aza-C was added to give a final concentration of $3 \mu\text{M}$. On the next day, the medium was

replaced with the fresh conditioned medium, followed by culturing for further 4 weeks. During this period, the medium was replaced with the fresh conditioned medium once 3 days. Thus, it was observed that myotubes derived from the bone marrow cell (BMSC) having the potential to differentiate into cardiomyocytes showed no increase but the expression of the two myocardium-specific genes (ANP and cTnI) was promoted by the addition of the conditioned medium. In case of the Nkx2.5 and GATA forced expressing bone marrow cells (BMSC-Nkx2.5-GATA4) having the potential to differentiate into cardiomyocytes, on the other hand, the myotubes showed no increase and the expression of the two myocardium-specific genes (ANP and cTnI) was promoted at the same level as in Nkx2.5 and GATA4 by the addition of the conditioned medium, showing no promoting effect.

Next, it was examined whether or not cardiomyocyte-expressing extracellular matrix (ECM) has an activity of promoting the differentiation into cardiomyocytes, culture dishes wherein cardiomyocytes had been cultured were treated with 0.45% trypsin/EDTA for about 30 minutes to eliminate the cardiomyocytes. Thus, culture dishes coated with the extracellular matrix of the cultured cardiomyocytes (hereinafter referred to as the "ECM-coated dishes") were prepared. Subsequently, bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes or compulsively both Nkx2.5 and GATA 4

genes-expressed bone marrow cells (BMSC-Nkx2.5-GATA4) having the potential to differentiate into cardiomyocytes were cultured in these 6-cm culture dishes at a density of 1×10^5 cells and then 5-aza-C was added to give a final concentration of 3 μM . On the next day, the medium was replaced with a fresh medium to eliminate 5-aza-C and the culture was continued for further 4 weeks. During this period, the medium was replaced with a fresh medium once 3 days. Thus, it was observed that myotubes derived from the bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes showed no increase but the expression of the two myocardium-specific genes (ANP and cTnI) was promoted by the coated dish. In case of the compulsively both Nkx2.5 and GATA4 genes-expressed bone marrow cells (BMSC-Nkx2.5-GATA4) having the potential to differentiate into cardiomyocytes, on the other hand, the myotubes showed no increase and the expression of the two myocardium-specific genes (ANP and cTnI) was promoted at the same level as in Nkx2.5 and GATA4 by the addition of the conditioned medium, showing no promoting effect.

Next, 2×10^4 cultured cardiomyocytes were co-cultured together with 8×10^4 bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes or 8×10^4 compulsively both Nkx2.5 and GATA4 genes-expressed bone marrow cells (BMSC-Nkx2.5-GATA4) having the potential to differentiate into cardiomyocytes in 6-cm culture dishes.

To distinguish the cultured cardiomyocytes from the bone marrow cells, the two types of bone marrow cells (BMSC and BMSC-Nkx2.5-GATA4) were labeled with GFP as in Example 5. On the next day after the co-culturing, 5-aza-C was added to give a final concentration of 3 μ M. On the next day, the medium was replaced with a fresh medium to eliminate 5-aza-C, followed by culturing for further 4 weeks. During this period, the medium was replaced with a fresh medium once 3 day. As a result, beating cardiomyocytes were increased about 10 times or more than the case wherein BMSC or BMSC-Nkx2.5-GATA4 were cultured alone. Thus, it was found that the efficiency of the differentiation into cardiomyocytes can be elevated 500 times or more by combining the forced expression of the Nkx2.5 and GATA4 genes with the co-culturing with cardiomyocytes.

Example 10

Analysis of surface antigens of KUM2 cells and BMSCs:

Surface antigens of KUM2 cells and BMSCs were analyzed to clearly differentiate KUM2 cells from BMSCs and develop a method for efficiently isolating and purifying cells having the potentiality of forming myocardium from bone marrow.

The surface antigens employed in the analysis included 20 antigens, i.e., CD105, Flk-1, CD31 and CD144 known as surface antigens of vascular endothelial cells,

CD34, CD117(c-kit), CD14, CD45, CD90, Ly6A/E(Sca-1), Ly6c and ly6g known as surface antigens in hematopoietic cells, CD140 known as surface antigens of mesenchymal cells, integrins CD49b, CD49d and CD29 and matrix receptors CD54, CD102, CD106 and CD44.

First, 1×10^4 KUM2 cells or 1×10^4 BMSC cells were pipetted into a 96-well U-shaped plate. An anti-mouse CD105 antibody (manufactured by Pharmingen), which had been biotin-labeled by a publicly known method (*Enzyme Antibody Technique*, Gakusai Kikaku (1985)), was added to a buffer for FACS (1% BSA-PBS, 0.02% EDTA, 0.05% NaN_3 , pH 7.4), then added to the wells and allowed to react on ice for 30 minutes. As a negative control, rat IgG2a, κ -purified antibody (manufactured by Pharmingen) was used. After washing with the buffer twice, 20 μl of streptoavidin-PE (manufactured by Nippon Becton Dickinson) was added. Then the mixture was allowed to react in the dark on ice for 30 minutes, washed with the buffer thrice and suspended in 500 μl of the buffer. The fluorescence intensity was measured with a flow cytometer and it was examined whether or not the fluorescence intensity was increased by adding the antibody. The results are shown in Fig. 1. As a result, it was found that the KUM2 cells and the BMSC cells were both CD105-negative.

Regarding the occurrence of the expression of the Flk-1 antigen, an antibody reaction was carried out in the

manner similar to that described above, using a biotinylated anti-mouse Flk-1 antibody (PM-28181D, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 2. As a result, it was found that the KUM2 cells and the BMSC cells were both Flk-1-negative.

Regarding the occurrence of the expression of the CD31 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD31 antibody (PM-01954D, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 3. As a result, it was found that the KUM2 cells and the BMSC cells were both CD31-negative.

Regarding the occurrence of the expression of the CD144 antigen, an antibody reaction was carried out using a biotinylated anti-mouse CD144 antibody (PM-28091D, manufactured by Pharmingen) followed by measurement with a flow cytometer. The results are shown in Fig. 4. As a result, it was found that the KUM2 cells were CD144-negative, while the BMSC cells were CD144-weak positive.

Regarding the occurrence of the expression of the CD34 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD34 antibody (PM-09434D, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 5. As a result, it was found that the KUM2 cells were CD34-negative,

while the BMSC cells were a mixture of CD34-positive cells and CD34-negative cells.

Regarding the occurrence of the expression of the CD117(c-kit) antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD117 antibody (PM-01904D, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 6. As a result, it was found that the KUM2 cells were CD117-negative, while the BMSC cells were CD117-positive.

Regarding the occurrence of the expression of the CD14 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD14 antibody (PM-09474, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 7. As a result, it was found that the KUM2 cells were CD14-positive, while the BMSC cells were CD14-negative.

Regarding the occurrence of the expression of the CD45 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD45 antibody (PM-01114, manufactured by Pharmingen), followed by the measurement with a flow cytometer. The results are shown in Fig. 8. As a result, it was found that the KUM2 cells and the BMSC cells were both CD45-negative.

Regarding the occurrence of the expression of the CD90 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD90 antibody (PM-22214,

manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 9. As a result, it was found that the KUM2 cells and the BMSC cells were both CD90-negative.

Regarding the occurrence of the expression of the Ly6A/E(Sca-1) antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse Ly6A/E(Sca-1) antibody (PM-01164A, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 10. As a result, it was found that the KUM2 cells and the BMSC cells were both Ly6A/E(Sca-1)-positive.

Regarding the occurrence of the expression of the Ly6c antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse Ly6c antibody (PM-01152, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 11. As a result, it was found that the KUM2 cells and the BMSC cells were both Ly6c-positive.

Regarding the occurrence of the expression of the Ly6g antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse Ly6g antibody (PM-01214, manufactured by Pharmingen), followed by the measurement with a flow cytometer. The results are shown in Fig. 12. As a result, it was found that the KUM2 cells and the BMSC cells were both Ly6g-negative.

Regarding the occurrence of the expression of the CD140 antigen, an antibody reaction was carried out using a biotinylated anti-mouse CD140 antibody (PM-28011A, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 13. As a result, it was found that the KUM2 cells and the BMSC cells were both CD140-positive.

Regarding the occurrence of the expression of the CD49b antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD49b antibody (PM-09794, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 14. As a result, it was found that the KUM2 cells were CD49b-positive, while the BMSC cells were CD49b-negative.

Regarding the occurrence of the expression of the CD49d antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD49d antibody (PM-01274, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 15. As a result, it was found that the KUM2 cells and the BMSC cells were both CD49d-negative.

Regarding the occurrence of the expression of the CD29 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD29 antibody (PM-22634, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 16. As a

result, it was found that the KUM2 cells and the BMSC cells were both CD29-positive.

Regarding the occurrence of the expression of the CD54 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD54 antibody (PM-01544, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 17. As a result, it was found that the KUM2 cells were CD54-positive, while the BMSC cells were CD54-negative.

Regarding the occurrence of the expression of the CD102 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD102 antibody (PM-01804, manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 18. As a result, it was found that the KUM2 cells and the BMSC cells were both CD102-negative.

Regarding the occurrence of the expression of the CD106 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD106 antibody (PM-01814 manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 19. As a result, it was found that the KUM2 cells were CD106-positive, while the BMSC cells were CD106-negative.

Regarding the occurrence of the expression of the CD44 antigen, an antibody reaction was carried out using an FITC-labeled anti-mouse CD44 antibody (PM-28154,

manufactured by Pharmingen), followed by measurement with a flow cytometer. The results are shown in Fig. 20. As a result, it was found that the KUM2 cells and the BMSC cells were both CD44-positive.

Table 1 shows the summarized analytical data obtained using the flow cytometer.

Table 1

	KUM2	BMSC
Hemato		
CD34	-	± ^{*1}
CD117(c-kit)	-	+
CD14	+	-
CD45	-	-
CD90(Thy1)	-	-
Ly-6a/e(Scal)	+	+
Ly6c	+	+
Ly6g	-	-
Endothelial		
Flk-1	-	-
CD31	-	-
CD105	-	-
CD144	-	+ ^{*2}
Mesenchyama		
CD140(PDGFR)	+	+
Integrin		
CD49b(α2)	+	-
CD49b(α4)	-	-
CD29(β1)	+	+
Matrix		
CD54(ICAM-1)	+	-
CD102(ICAM-2)	-	-
CD106(VCAM-1)	+	-
CD44(Hyaluronate)	+	+

1: mixture; 2: weak positive

Example 11

Concentration of differentiation precursor cells using mouse MLC2v promoter:

In order to efficiently obtain cells having the potential to differentiate into myocardium from mouse bone marrow-derived cells having the potential to differentiate into cardiomyocytes, a promoter expression system of a mouse MLC2v (myosin light chain-2v) gene showing cardiomyocyte-specific expression was constructed. Specifically, an EGFP gene (manufactured by CLONTECH) was ligated to the downstream of the promoter sequence of the mouse MLC2v gene followed by constructing a pMLC-2-EGFP plasmid containing the expression unit of neomycin-resistance gene. DNA of this plasmid was obtained by the alkali neutralization method described in *Molecular Cloning, A Laboratory Manual*, 2nd ed. etc.

2 μ g of the above-described DNA was introduced using LIPOFECTAMINE (manufactured by LIFE TECHNOLOGY) into KUM2 cells, which had been cultured in a 6-well plate to give 1×10^5 cells. Detailed procedure was carried out in accordance with the manufacturer's instructions. Forty-eight hours after the gene transfection, G418 (manufactured by Sigma) was added to give a final concentration of 1 mg/ml followed by selecting survived cells which were transfected by the gene.

On the 14th day after the gene introduction, 5-aza-C was added to give a final concentration of 3 μ M, and 24 hours thereafter, the medium was replaced and the differentiation was induced. From the day 3 after the induction of the differentiation, GFP-positive cells were observed. On the day 4 after the induction of the differentiation, GFP-positive cells were exclusively selected from among 1×10^4 cells using an FACS Caliber (manufactured by Becton Dickinson) and the culturing was further continued. As a result, 90% or more cells had differentiated into cells having a myotube-like structure, which indicates that cells with differentiation potency could be efficiently concentrated. After collecting by FACS, these GFP-positive cells were transplanted in accordance with the method of Example 10. As a result, these cells differentiated not into hemoendothelium but specifically into muscle tissues such as skeletal muscle and myocardium.

Example 12

Induction of adipocytes from mouse bone marrow-derived cells having the potential to differentiate into cardiomyocytes:

Bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes can be induced to differentiate not only into cardiomyocytes but also into

adipocytes. To control the differentiation into adipocytes, the conditions for the induction of the differentiation were examined. First, the expression of PPAR- γ receptors was analyzed by the quantitative PCR method. As a result, it was found that PPAR- γ 1 receptor was expressed but PPAR γ 2 receptor was not expressed in the BMSCs. Subsequently, PPAR- γ receptor agonists, pioglitazone and troglitazone, were added at various concentrations to bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes. As a result, the differentiation into adipocytes was promoted, depending on the concentration, and about 50% and 100% of the BMSCs differentiated into adipocytes respectively at 0.4 μ M and 2 μ M.

Example 13

Induction of differentiation into neurocytes, hepatocytes and cardiomyocytes of mouse bone marrow-derived cells having the potential to differentiate into cardiomyocytes by transplantation into blastocysts:

In order to obtain stable transformants of GFP-labeled bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes, gene transfection was first performed in the following manner.

GFP was introduced into a retrovirus vector plasmid pCLNCX (manufactured by Imgenex) to prepare pCLNC-GFP. The retrovirus vector plasmid pCLNC-GFP and a pCMV-Eco plasmid

vector (manufactured by Imgenex) capable of expressing an ecotropic gene were treated by the alkali neutralization method and the PEG precipitation method described in *Molecular Cloning, A Laboratory Manual*, 2nd ed. etc. to obtain DNAs of high purity.

A day before the transfection of these DNAs, 293 cells carrying gag and pol genes, which had become confluent were subcultured into a 10 cm dish by a dilution ratio of 1/5 and cultured at 37°C in a 5% CO₂-incubator.

The transfection was carried out in the following manner.

In 0.5 ml of 250 mM CaCl₂ (pH 6.95), 5 µg of the pCLNC-GFP retrovirus vector plasmid DNA and 5 µg of the pCMV-Eco plasmid vector DNA were dissolved. The solution thus obtained was dropped into 0.5 ml of 2× BBS (50 mM BES (N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid), 280 mM NaCl, 1.5 mM Na₂HPO₄ (pH 6.95)) in a 15 ml tube and allowed to stand at room temperature for 10 minutes. Subsequently, the DNA solution was dropped into the medium of the 293 cells prepared on the previous day and cultured at 37°C in a 5% CO₂-incubator. On the next day, the medium was replaced and culture was further continued at 37°C in a 5% CO₂-incubator.

Two days after the replacement of the medium, the culture supernatant was filtered through a 0.45 µm filter

(manufactured by Millipore) and a solution containing the virus vector was collected.

The mouse bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes, into which the virus vector was introduced, were plated into a 6-well dish at a density of 2×10^4 cells/well on the previous day of the infection with the virus.

Hexadimethrine bromide (polybrene) (manufactured by Sigma) was added to the virus vector-containing solution obtained above to give a final concentration of 8 $\mu\text{g/ml}$. After replacing by the medium of the mouse bone marrow cells (BMSC) having the potential to differentiate into cardiomyocytes, followed by culturing at 33°C in a 5% CO_2 -incubator. Five hours thereafter, the medium was replaced with fresh IMDM, followed by further culturing at 33°C in a 5% CO_2 -incubator.

After culturing for two days, G418 was added until the final concentration of G418 came to be 300 $\mu\text{g/ml}$, followed by further culturing for further 7 days. During this period, a part of the cells died to be suspended. The surviving cells were suspended with trypsin and scattered in a fresh culture dish.

The obtained GFP-labeled bone marrow-derived cells having the potential to differentiate into cardiomyocytes were grown in a 6-cm culture dish. After eliminating the medium, 0.5 ml of 0.25% trypsin EDTA was added and the

treatment was carried out for 1 minute. Then, 1.5 ml of a fresh medium was added and the cells were suspended. After adding fetal bovine serum (manufactured by Lexicon Genetics) and mixing, the cell suspension was poured into mouse blastocyst. The mouse blastocysts were obtained by spontaneously mating female C57Bl/6J mice subjected to hyper-ovulation with male mice of the same line, taking out the uterus 4 days thereafter, and perfusing the inside of the uterus with M15 medium. After allowing to stand at 37°C under 5% CO₂ until the blastocyst cavities sufficiently dilated, the blastocyst were transferred into M15 medium containing 20 mM HEPES which was cooled to about 4°C. Then, 10 to 15 BMSCs were microscopically injected into each blastocyst cavity while observing under an inverted microscope (manufactured by Nikon) provided with a microinjector (manufactured by Narumo Kagaku) and a micromanipulator (manufactured by Narumo Kagaku). After allowing to stand at 37°C under 5% CO₂ until the blastocyst cavities sufficiently dilated, the blastocysts were transplanted into the oviducal side of the uterus of female MCH mice with pseudopregnancy, followed by implantation. The female MCH mice with pseudopregnancy were prepared by mating with vasoligated male MCH mice aged 10 weeks or more on 17:00 three days before the transplantation at the ratio of 1:1. On 9:00 on the next morning, vaginal plugs were

confirmed, and two days thereafter, the female mice were used for the above-described purpose.

The mice thus born were sacrificed and organs were extirpated for observing the expression of GFP. As a result, the expression of GFP was observed in the brain and the liver, which suggested that the BMSCs had differentiated into the nerve system and the liver. Genomic DNA was obtained from the heart taken out from another individual and subjected to PCR using the primers of SEQ ID NOS:79 and 80. As a result, it was confirmed that BMSCs were also incorporated into the heart. These results indicate that BMSCs have a totipotency of differentiating into all of the three germ layers of nerve, heart and liver.

Example 14

Telomerase activity in mouse bone marrow cells having the potential to differentiate into cardiomyocytes:

The mouse bone marrow cells having the potential to differentiate into cardiomyocytes were examined for telomerase activity by the Telomeric Repeat Amplification Protocol (TRAP) method (TRAPEze Telomerase Detection kit, manufactured by Oncor). The measurement of the telomerase activity was carried out as described below according to, in principle, the manufacture's instructions. The mouse bone marrow cells having the potential to differentiate

into cardiomyocytes which had been cultured in a 6-cm culture dish (about 10^6 cells) were washed with PBS, followed by addition of 200 μ l of 1x CHAPS solution. After being allowed to stand on ice for 30 minutes, the cells were recovered together with the solution to a 1.5 ml centrifuge tube and centrifuged at 14000 rpm for 20 minutes (4°C; himac CF15, manufactured by Hitachi, Ltd.). The supernatant was recovered as a cell extract and the protein content was determined using Protein Assay (manufactured by BioRad). The protein content of the cell extract made from the mouse bone marrow cells having the potential to differentiate into cardiomyocytes under the above conditions was found to be about 1 mg/ml.

The cell extract was then subjected to telomerase elongation reaction and PCR amplification according to the manufacture's instructions. As the Taq polymerase, EX Taq polymerase (manufactured by Takara Shuzo) was used. After completion of the reactions, the samples were mixed with a 1/10 volume of 10x staining solution (0.25% bromophenol blue, 0.25% xylene cyanol FF, and 30% glycerol) and subjected to electrophoresis on 12.5% polyacrylamide gel (prepared according to the manufacture's instructions of TRAPeze Telomerase Detection Kit) at a constant voltage of 250 mV. After the electrophoresis, the gel was stained with Cyber Green (FMC) and analyzed using a fluorescence image analyzer, FluoroImager (manufactured by Molecular

Dynamics). The telomerase activity was detected in the samples of the cell extracts having final concentration of 0.4-4 µg/ml.

Example 15

Isolation and culturing of bone marrow cell having the potential to differentiate into cardiomyocyte from rat bone marrow:

Six female Wistar rats of five week age (SLC Japan) were subjected to cervical dislocation and then disinfected by sufficiently applying 70% ethanol. Next, the skin of each leg was incised in a broad range and muscles covering the thighbone and shinbone were cut out to obtain the thighbone and shinbone. The thus obtained thighbone and shinbone were transferred into a culture dish of 10 cm in diameter (manufactured by Iwaki Glass) filled with PBS (manufactured by Gibco BRL) and muscles and joints were completely removed. Next, both ends of these bones were cut out using scissors, and the contents of bone marrow were squeezed out with a water flow of a culture liquid (D-PBS, manufactured by Gibco BRL) using a 10 ml syringe (manufactured by Terumo) equipped with a 20G needle. The thus obtained cell mass was loosened into a homogeneous level by passing through the syringe. The thus obtained cell suspension was recovered into a 50 ml capacity centrifugation tube (manufactured by BECTON DICKINSON) and

centrifuged at 1,500 rpm for 10 minutes (a low speed centrifuge manufactured by TOMY), and the precipitated cells were suspended in 6 ml of D-PBS. When the number of cells was counted using a modified Neubauer counting chamber, the recovered cells were 2.6×10^9 in total. This result means that 1×10^8 cells were recovered from one thighbone or shinbone. The thus recovered cells were diluted to a density of 1.3×10^8 cells per 1 ml, 5 ml of the resulting suspension was overlaid on a 1.073 g/ml Percoll (manufactured by Amersham Pharmacia Biotech)/D-PBS solution (25 ml) which had been put into a 50 ml capacity centrifugation tube, followed by centrifugation at room temperature and at 3,100 rpm for 30 minutes. After the centrifugation, cells were recovered from the interface between the Percoll solution and cell suspension, diluted to 4 times with D-PBS and centrifuged at 2,300 rpm for 10 minutes and then the thus fractionated cells were recovered. The thus recovered cells were suspended in IMDM medium (manufactured by Gibco BRL) containing 20% FCS, 100 μ g/ml penicillin, 250 ng/ml streptomycin and 85 μ g/ml amphotericin (manufactured by Gibco BRL). When the number of cells at this stage was again counted, the recovered bone marrow cells were 4.7×10^7 in total, meaning that cells corresponding to about 2% of the cells before the treatment were recovered. The fractionated bone marrow cells were plated on three culture dishes for animal cells having a

diameter of 10 cm (manufactured by Iwaki Glass, hereinafter referred to as "10-cm culture dish") to a density of 2 to 5×10^5 cells/cm² and cultured at 33°C in a 5% CO₂-incubator (manufactured by Tabai). A half volume of the medium was exchanged with a fresh medium after 24 hours and 72 hours. Three or 4 days thereafter, a half volume of the medium was exchanged with a fresh medium. Since colonies became dense after a lapse of 15 days, the cells were removed with a trypsin EDTA treatment and a 2/3 part of them was suspended in 4 ml of a stock solution (10% DMSO, 50% bone marrow cell culture supernatant and 40% the above medium which had not been used), dispensed in 1 ml portions into 2 ml capacity tubes (manufactured by Sumitomo Bakelite) and stored in a freezer, and the remaining 1/3 part was again inoculated into two 10-cm culture dishes and subcultured.

Example 16

Evaluation of rat bone marrow-derived cell having the potential to differentiate into cardiomyocyte:

The rat bone marrow cells subcultured in the above were again removed with the trypsin EDTA treatment when they became dense and inoculated into a 6 well plate (manufactured by BECTON DICKINSON) in 5×10^4 cells per well or into a 6 cm diameter culture dish coated with human fibronectin (Biocoat, manufactured by BECTON DICKINSON) in a density of 1.3×10^5 cells. One day thereafter, culturing

was carried out under two different conditions, one in which only 5-azacytidine (manufactured by Sigma, 10 μ M in final concentration) was added, and another in which 5-azacytidine, PDGF-BB (manufactured by Pepro Tech EC LTD, 10 ng/ml in final concentration) and all-trans retinoic acid (RA, manufactured by Sigma, 10^{-9} M in final concentration) were added, and the medium was exchanged after 2 days of the culturing (in the latter conditions, PDGF and all-trans retinoic acid were again added at the time of the medium exchange and after 2 days and 4 days). Three or 4 days thereafter, the medium was exchanged, followed by culturing for 3 weeks. As a result, differentiation of myotube-like cells was observed in the conditions in which 5-azacytidine, PDGF-BB and retinoic acid were added.

Example 17

Forced expression of transcription factor MesP1 and enhancement of cardiomyocyte differentiation by addition of cytokine:

Influences of forced expression of a cardiomyocyte differentiation-related transcription factor MesP1 in a bone marrow-derived pluripotent stem cell (BMSC) having the potential to differentiate into cardiomyocytes upon its differentiation into cardiomyocytes and influences of a combination of forced expression of MesP1 with cytokine

(FGF-8, ET-1, Midkine or BMP4) upon differentiation into cardiomyocytes were examined.

A mouse bone marrow-derived pluripotent stem cell (BMSC-MesP1) having the potential to differentiate into cardiomyocytes in which the MesP1 gene was forced-expressed was obtained using a retrovirus vector in the same manner as in Example 6, and then the differentiation was induced to examine efficiency of differentiation into cardiomyocytes.

The bone marrow cell (BMSC-MesP1) having the potential to differentiate into cardiomyocytes in which MesP1 was forced expressed was plated into a 60-mm culture dish in a density of 2×10^4 cells/ml and cultured at 33°C in a 5% CO₂-incubator. On the next day, 5-aza-C was added to the culture medium to give a final concentration of 3 μM, followed by culturing under five different conditions, namely (i) addition of FGF-8 to give a final concentration of 10 ng/ml (culture dish N), (ii) addition of ET-1 to give a final concentration of 10 ng/ml (culture dish P), (iii) addition of Midkine to give a final concentration of 10 ng/ml (culture dish Q), (iv) addition of BMP4 to give a final concentration of 10 ng/ml (culture dish R), and (v) no addition (culture dish S).

On the next day, the medium was exchanged with a fresh medium in order to eliminate 5-aza-C from the medium, and then the culturing was continued by adding FGF-8 to the

culture dish N to give a final concentration of 10 ng/ml, ET-1 to the culture dish P to give a final concentration of 10 ng/ml, Midkine to the culture dish Q to give a final concentration of 10 ng/ml and BMP4 to the culture dish R to give a final concentration of 10 ng/ml. Two days and 4 days thereafter, the medium exchange and addition of FGF-8, ET-1, Midkine or BMP4 were carried out similarly.

Four weeks after the addition of 5-aza-C, morphology of the cells was observed under a phase contrast microscope. As a result, the number of myotube-like cells was not changed greatly by the forced expression of MesP1. In addition, about 50% of the cells became myotube-like cells in the culture dish to which FGF-8, ET-1, Midkine or BMP4 had been added.

Next, RNA was recovered from the thus obtained myotube-like cells, and genes expressing in the myotube-like cells were analyzed by quantitative PCR using the synthetic oligonucleotides shown in SEQ ID NOS:71 to 78. As a result, expression of ANP as a gene specific for a myocardium was accelerated by the forced expression of MesP1. On the other hand, FGF-8, ET-1, Midkine or BMP4 did not further accelerate the expression of ANP accelerated by the forced expression of MesP1.

INDUSTRIAL APPLICABILITY

The present invention provides a bone marrow cell, a growth factor, a vitamin and an adhesion molecule which are effective for treating a heart disease accompanied with destruction and denaturation of a cardiomyocyte and for screening a therapeutic agent for it, and application methods thereof.

FREE TEXT OF SEQUENCE LISTINGS:

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EDITORIAL NOTE

APPLICATION NUMBER – 22281/01

The following sequence listing pages 1-90 are part of the description. The claims pages follow on pages 143-146

SEQUENCE LISTING

SEQUENCING LISTING

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CARDIOMYOCYTES

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Lys Pro Lys Leu Lys	Glu Val Gln Val	Arg Leu Glu Glu His Leu Glu		
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Cys Ala Cys Ala Thr	Thr Ser Leu Asn	Pro Asp Tyr Arg Glu Glu Asp		
	180	185		190
Thr Asp Val Arg				
	195			

<210> 4

<211> 588

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(591)

<400> 4

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Met Arg Thr Leu Ala Cys	Leu Leu Leu Leu Gly Cys	Gly Tyr Leu Ala	
1	5	10	15
cat gtt ctg gcc gag gaa	gcc gag atc ccc cgc	gag gtg atc gag agg	96
His Val Leu Ala Glu Glu	Ala Glu Ile Pro Arg	Glu Val Ile Glu Arg	
	20	25	30
ctg gcc cgc agt cag atc	cac agc atc cgg gac	ctc cag cga ctc ctg	144
Leu Ala Arg Ser Gln Ile	His Ser Ile Arg Asp	Leu Gln Arg Leu Leu	
	35	40	45

gag ata gac tcc gta ggg agt gag gat tct ttg gac acc agc ctg aga	192
Glu Ile Asp Ser Val Gly Ser Glu Asp Ser Leu Asp Thr Ser Leu Arg	
50 55 60	
gct cac ggg gtc cac gcc act aag cat gtg ccc gag aag cgg ccc ctg	240
Ala His Gly Val His Ala Thr Lys His Val Pro Glu Lys Arg Pro Leu	
65 70 75 80	
ccc att cgg agg aag aga agc atc gag gaa gct gtc ccc gct gtc tgc	288
Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Val Pro Ala Val Cys	
85 90 95	
aag acc agg acg gtc att tac gag att cct cgg agt cag gtc gac ccc	336
Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro	
100 105 110	
acg tcc gcc aac ttc ctg atc tgg ccc ccg tgc gtg gag gtg aaa cgc	384
Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg	
115 120 125	
tgc acc ggc tgc tgc aac acg agc agt gtc aag tgc cag ccc tcc cgc	432
Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg	
130 135 140	
gtc cac cac cgc agc gtc aag gtg gcc aag gtg gaa tac gtc agg aag	480
Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys	
145 150 155 160	
aag cca aaa tta aaa gaa gtc cag gtg agg tta gag gag cat ttg gag	528
Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu	
165 170 175	
tgc gcc tgc gcg acc aca agc ctg aat ccg gat tat cgg gaa gag gac	576
Cys Ala Cys Ala Thr Thr Ser Leu Asn Pro Asp Tyr Arg Glu Glu Asp	
180 185 190	
acg gat gtg agg	588
Thr Asp Val Arg	
195	
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<211> 241	
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Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg	

1	5	10	15
Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met			
20	25	30	
Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu			
35	40	45	
His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met			
50	55	60	
Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg			
65	70	75	80
Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu			
85	90	95	
Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp			
100	105	110	
Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln			
115	120	125	
Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr			
130	135	140	
Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg			
145	150	155	160
Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu			
165	170	175	
Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser			
180	185	190	
Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val			
195	200	205	
Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg			
210	215	220	
Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly			
225	230	235	240

Ala

<210> 6

<211> 723

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(726)

<400> 6

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Met Asn Arg Cys Trp Ala Leu Phe Leu Ser Leu Cys Cys Tyr Leu Arg	
1 5 10 15	
ctg gtc agc gcc gag ggg gac ccc att ccc gag gag ctt tat gag atg	96
Leu Val Ser Ala Glu Gly Asp Pro Ile Pro Glu Glu Leu Tyr Glu Met	
20 25 30	
ctg agt gac cac tcg atc cgc tcc ttt gat gat ctc caa cgc ctg ctg	144
Leu Ser Asp His Ser Ile Arg Ser Phe Asp Asp Leu Gln Arg Leu Leu	
35 40 45	
cac gga gac ccc gga gag gaa gat ggg gcc gag ttg gac ctg aac atg	192
His Gly Asp Pro Gly Glu Glu Asp Gly Ala Glu Leu Asp Leu Asn Met	
50 55 60	
acc cgc tcc cac tct gga ggc gag ctg gag agc ttg gct cgt gga aga	240
Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg	
65 70 75 80	
agg agc ctg ggt tcc ctg acc att gct gag ccg gcc atg atc gcc gag	288
Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu	
85 90 95	
tgc aag acg cgc acc gag gtg ttc gag atc tcc cgg cgc ctc ata gac	336
Cys Lys Thr Arg Thr Glu Val Phe Glu Ile Ser Arg Arg Leu Ile Asp	
100 105 110	
cgc acc aac gcc aac ttc ctg gtg tgg ccg ccc tgt gtg gag gtg cag	384
Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln	
115 120 125	
cgc tgc tcc ggc tgc tgc aac aac cgc aac gtg cag tgc cgc ccc acc	432
Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr	
130 135 140	
cag gtg cag ctg cga cct gtc cag gtg aga aag atc gag att gtg cgg	480
Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg	
145 150 155 160	
aag aag cca atc ttt aag aag gcc acg gtg acg ctg gaa gac cac ctg	528
Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu	
165 170 175	
gca tgc aag tgt gag aca gtg gca gct gca cgg cct gtg acc cga agc	576

Ala Cys Lys Cys Glu Thr Val	Ala Ala Ala Arg Pro Val Thr Arg Ser	
180	185	190
ccg ggg ggt tcc cag gag cag cga gcc aaa acg ccc caa act cgg gtg		624
Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val		
195	200	205
acc att cgg acg gtg cga gtc cgc cgg ccc ccc aag ggc aag cac cgg		672
Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg		
210	215	220
aaa ttc aag cac acg cat gac aag acg gca ctg aag gag acc ctt gga		720
Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly		
225	230	235
		240
gcc		723
Ala		
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<212> PRT		
<213> Homo sapiens		
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Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala Leu Pro Glu Asp Gly		
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		15
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu		
20	25	30
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg		
35	40	45
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu		
50	55	60
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn		
65	70	75
		80
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys		
85	90	95
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr		
100	105	110
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys		
115	120	125
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys		
130	135	140

Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser

145

150

<210> 8

<211> 465

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(468)

<400> 8

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1 5 10 15	
ggc agc ggc gcc ttc ccg ccc ggc cac ttc aag gac ccc aag cgg ctg	96
Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys Asp Pro Lys Arg Leu	
20 25 30	
tac tgc aaa aac ggg ggc ttc ttc ctg cgc atc cac ccc gac ggc cga	144
Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile His Pro Asp Gly Arg	
35 40 45	
gtt gac ggg gtc cgg gag aag agc gac cct cac atc aag cta caa ctt	192
Val Asp Gly Val Arg Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu	
50 55 60	
caa gca gaa gag aga gga gtt gtg tct atc aaa gga gtg tgt gct aac	240
Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn	
65 70 75 80	
cgt tac ctg gct atg aag gaa gat gga aga tta ctg gct tct aaa tgt	288
Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys	
85 90 95	
gtt acg gat gag tgt ttc ttt ttt gaa cga ttg gaa tct aat aac tac	336
Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr	
100 105 110	
aat act tac cgg tca agg aaa tac acc agt tgg tat gtg gca ttg aaa	384
Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys	
115 120 125	
cga act ggg cag tat aaa ctt gga tcc aaa aca gga cct ggg cag aaa	432
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln Lys	

130 135 140
 gct ata ctt ttt ctt cca atg tct gct aag agc 465
 Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
 145 150 155
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 <211> 324
 <212> PRT
 <213> Homo sapiens
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 1 5 10 15
 Ile Leu Asn Leu Glu Gln Gln Gln Arg Ser Leu Ala Ala Ala Gly Glu
 20 25 30
 Leu Ser Ala Arg Leu Glu Ala Thr Leu Ala Pro Ser Ser Cys Met Leu
 35 40 45
 Ala Ala Phe Lys Pro Glu Ala Tyr Ala Gly Pro Glu Ala Ala Ala Pro
 50 55 60
 Gly Leu Pro Glu Leu Arg Ala Glu Leu Gly Arg Ala Pro Ser Pro Ala
 65 70 75 80
 Lys Cys Ala Ser Ala Phe Pro Ala Ala Pro Ala Phe Tyr Pro Arg Ala
 85 90 95
 Tyr Ser Asp Pro Asp Pro Ala Lys Asp Pro Arg Ala Glu Lys Lys Glu
 100 105 110
 Leu Cys Ala Leu Gln Lys Ala Val Glu Leu Glu Lys Thr Glu Ala Asp
 115 120 125
 Asn Ala Glu Arg Pro Arg Ala Arg Arg Arg Arg Lys Pro Arg Val Leu
 130 135 140
 Phe Ser Gln Ala Gln Val Tyr Glu Leu Glu Arg Arg Phe Lys Gln Gln
 145 150 155 160
 Arg Tyr Leu Ser Ala Pro Glu Arg Asp Gln Leu Ala Ser Val Leu Lys
 165 170 175
 Leu Thr Ser Thr Gln Val Lys Ile Trp Phe Gln Asn Arg Arg Tyr Lys
 180 185 190
 Cys Lys Arg Gln Arg Gln Asp Gln Thr Leu Glu Leu Val Gly Leu Pro
 195 200 205
 Pro Pro Pro Pro Pro Pro Ala Arg Arg Ile Ala Val Pro Val Leu Val

210	215	220
Arg Asp Gly Lys Pro Cys Leu Gly Asp Ser Ala Pro Tyr Ala Pro Ala		
225	230	235
Tyr Gly Val Gly Leu Asn Pro Tyr Gly Tyr Asn Ala Tyr Pro Ala Tyr		240
	245	250
Pro Gly Tyr Gly Gly Ala Ala Cys Ser Pro Gly Tyr Ser Cys Thr Ala		255
	260	265
Ala Tyr Pro Ala Gly Pro Ser Pro Ala Gln Pro Ala Thr Ala Ala Ala		270
	275	280
Asn Asn Asn Phe Val Asn Phe Gly Val Gly Asp Leu Asn Ala Val Gln		285
	290	295
Ser Pro Gly Ile Pro Gln Ser Asn Ser Gly Val Ser Thr Leu His Gly		300
305	310	315
Ile Arg Ala Trp		320

<210> 10

<211> 972

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(975)

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Met Phe Pro Ser Pro Ala Leu Thr Pro Thr Pro Phe Ser Val Lys Asp	
1 5 10 15	
atc cta aac ctg gaa cag cag cag cgc agc ctg gct gcc gcc gga gag	96
Ile Leu Asn Leu Glu Gln Gln Gln Arg Ser Leu Ala Ala Ala Gly Glu	
20 25 30	
ctc tct gcc cgc ctg gag gcg acc ctg gcg ccc tcc tcc tgc atg ctg	144
Leu Ser Ala Arg Leu Glu Ala Thr Leu Ala Pro Ser Ser Cys Met Leu	
35 40 45	
gcc gcc ttc aag cca gag gcc tac gct ggg ccc gag gcg gct gcg ccg	192
Ala Ala Phe Lys Pro Glu Ala Tyr Ala Gly Pro Glu Ala Ala Ala Pro	
50 55 60	
ggc ctc cca gag ctg cgc gca gag ctg ggc cgc gcg cct tca ccg gcc	240

Gly	Leu	Pro	Glu	Leu	Arg	Ala	Glu	Leu	Gly	Arg	Ala	Pro	Ser	Pro	Ala		
65					70				75						80		
aag	tgt	gcg	tct	gcc	ttt	ccc	gcc	gcc	ccc	gcc	ttc	tat	cca	cgt	gcc	288	
Lys	Cys	Ala	Ser	Ala	Phe	Pro	Ala	Ala	Pro	Ala	Phe	Tyr	Pro	Arg	Ala		
			85						90					95			
tac	agc	gac	ccc	gac	cca	gcc	aag	gac	cct	aga	gcc	gaa	aag	aaa	gag	336	
Tyr	Ser	Asp	Pro	Asp	Pro	Ala	Lys	Asp	Pro	Arg	Ala	Glu	Lys	Lys	Glu		
			100					105					110				
ctg	tgc	gcg	ctg	cag	aag	gcg	gtg	gag	ctg	gag	aag	aca	gag	gcg	gac	384	
Leu	Cys	Ala	Leu	Gln	Lys	Ala	Val	Glu	Leu	Glu	Lys	Thr	Glu	Ala	Asp		
		115					120					125					
aac	gcg	gag	cgg	ccc	cgg	gcg	cga	cgg	cgg	agg	aag	ccg	cgc	gtg	ctc	432	
Asn	Ala	Glu	Arg	Pro	Arg	Ala	Arg	Arg	Arg	Arg	Lys	Pro	Arg	Val	Leu		
	130					135				140							
ttc	tgc	cag	gcg	cag	gtc	tat	gag	ctg	gag	cgg	cgc	ttc	aag	cag	cag	480	
Phe	Ser	Gln	Ala	Gln	Val	Tyr	Glu	Leu	Glu	Arg	Arg	Phe	Lys	Gln	Gln		
	145			150				155				160					
cgg	tac	ctg	tgc	gcc	ccc	gaa	cgc	gac	cag	ctg	gcc	agc	gtg	ctg	aaa	528	
Arg	Tyr	Leu	Ser	Ala	Pro	Glu	Arg	Asp	Gln	Leu	Ala	Ser	Val	Leu	Lys		
			165					170				175					
ctc	acg	tcc	acg	cag	gtc	aag	atc	tgg	ttc	cag	aac	cgg	cgc	tac	aag	576	
Leu	Thr	Ser	Thr	Gln	Val	Lys	Ile	Trp	Phe	Gln	Asn	Arg	Arg	Tyr	Lys		
			180					185				190					
tgc	aag	cgg	cag	cgg	cag	gac	cag	act	ctg	gag	ctg	gtg	ggg	ctg	ccc	624	
Cys	Lys	Arg	Gln	Arg	Gln	Asp	Gln	Thr	Leu	Glu	Leu	Val	Gly	Leu	Pro		
		195					200					205					
ccg	ccg	ccg	ccg	ccg	cct	gcc	cgc	agg	atc	gcg	gtg	cca	gtg	ctg	gtg	672	
Pro	Pro	Pro	Pro	Pro	Pro	Ala	Arg	Arg	Ile	Ala	Val	Pro	Val	Leu	Val		
		210				215					220						
cgc	gat	ggc	aag	cca	tgc	cta	ggg	gac	tgc	gcg	ccc	tac	gcg	cct	gcc	720	
Arg	Asp	Gly	Lys	Pro	Cys	Leu	Gly	Asp	Ser	Ala	Pro	Tyr	Ala	Pro	Ala		
	225			230				235				240					
tac	ggc	gtg	ggc	ctc	aat	ccc	tac	ggg	tat	aac	gcc	tac	ccc	gcc	tat	768	
Tyr	Gly	Val	Gly	Leu	Asn	Pro	Tyr	Gly	Tyr	Asn	Ala	Tyr	Pro	Ala	Tyr		
			245					250				255					
ccg	ggg	tac	ggc	ggc	gcg	gcc	tgc	agc	cct	ggc	tac	agc	tgc	act	gcc	816	

Pro Gly Tyr Gly Gly Ala Ala Cys Ser Pro Gly Tyr Ser Cys Thr Ala	
260	265
270	
gct tac ccc gcc ggg cct tcc cca gcg cag ccg gcc act gcc gcc gcc	864
Ala Tyr Pro Ala Gly Pro Ser Pro Ala Gln Pro Ala Thr Ala Ala Ala	
275	280
285	
aac aac aac ttc gtg aac ttc ggc gtc ggg gac ttg aat gcg gtt cag	912
Asn Asn Asn Phe Val Asn Phe Gly Val Gly Asp Leu Asn Ala Val Gln	
290	295
300	
agc ccc ggg att ccg cag agc aac tcg gga gtg tcc acg ctg cat ggt	960
Ser Pro Gly Ile Pro Gln Ser Asn Ser Gly Val Ser Thr Leu His Gly	
305	310
315	320
atc cga gcc tgg	972
Ile Arg Ala Trp	
324	
<210> 11	
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<400> 11	
Met Tyr Gln Ser Leu Ala Met Ala Ala Asn His Gly Pro Pro Pro Gly	
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10	15
Ala Tyr Gln Ala Gly Gly Pro Gly Pro Phe Met His Gly Ala Gly Ala	
20	25
30	
Ala Ser Ser Pro Val Tyr Leu Pro Thr Pro Arg Val Pro Ser Ser Val	
35	40
45	
Leu Gly Leu Ser Tyr Leu Gln Gly Gly Gly Ala Gly Ser Ala Ser Gly	
50	55
60	
Gly Pro Ser Gly Gly Ser Pro Gly Gly Ala Ala Ser Gly Ala Gly Pro	
65	70
75	80
Gly Thr Gln Gln Gly Ser Pro Gly Trp Ser Gln Ala Gly Ala Thr Gly	
85	90
95	
Ala Ala Tyr Thr Pro Pro Pro Val Ser Pro Arg Phe Ser Phe Pro Gly	
100	105
110	
Thr Thr Gly Ser Leu Ala Ala Ala Ala Ala Ala Ala Arg Glu	
115	120
125	
Ala Ala Ala Tyr Ser Ser Gly Gly Gly Ala Ala Gly Ala Gly Leu Ala	

130	135	140
Gly Arg Glu Gln Tyr Gly Arg Ala Gly Phe Ala Gly Ser Tyr Ser Ser		
145	150	155
Pro Tyr Pro Ala Tyr Met Ala Asp Val Gly Ala Ser Trp Ala Ala Ala		160
	165	170
Ala Ala Ala Ser Ala Gly Pro Phe Asp Ser Pro Val Leu His Ser Leu		175
	180	185
Pro Gly Arg Ala Asn Pro Ala Ala Arg His Pro Asn Leu Asp Met Phe		190
	195	200
Asp Asp Phe Ser Glu Gly Arg Glu Cys Val Asn Cys Gly Ala Met Ser		205
	210	215
Thr Pro Leu Trp Arg Arg Asp Gly Thr Gly His Tyr Leu Cys Asn Ala		220
225	230	235
Cys Gly Leu Tyr His Lys Met Asn Gly Ile Asn Arg Pro Leu Ile Lys		240
	245	250
Pro Gln Arg Arg Leu Ser Ala Ser Arg Arg Val Gly Leu Ser Cys Ala		255
	260	265
Asn Cys Gln Thr Thr Thr Thr Thr Leu Trp Arg Arg Asn Ala Glu Gly		270
	275	280
Glu Pro Val Cys Asn Ala Cys Gly Leu Tyr Met Lys Leu His Gly Val		285
	290	295
Pro Arg Pro Leu Ala Met Arg Lys Glu Gly Ile Gln Thr Arg Lys Arg		300
305	310	315
Lys Pro Lys Asn Leu Asn Lys Ser Lys Thr Pro Ala Ala Pro Ser Gly		320
	325	330
Ser Glu Ser Leu Pro Pro Ala Ser Gly Ala Ser Ser Asn Ser Ser Asn		335
	340	345
Ala Thr Thr Ser Ser Ser Glu Glu Met Arg Pro Ile Lys Thr Glu Pro		350
	355	360
Gly Leu Ser Ser His Tyr Gly His Ser Ser Ser Val Ser Gln Thr Phe		365
	370	375
Ser Val Ser Ala Met Ser Gly His Gly Pro Ser Ile His Pro Val Leu		380
385	390	395
Ser Ala Leu Lys Leu Ser Pro Gln Gly Tyr Ala Ser Pro Val Ser Gln		400
	405	410
Ser Pro Gln Thr Ser Ser Lys Gln Asp Ser Trp Asn Ser Leu Val Leu		415

420 425 430
 Ala Asp Ser His Gly Asp Ile Ile Thr Ala
 435 440
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 <400> 12
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 1 5 10 15
 gcc tac cag gcg ggc ggc ccc ggc ccc ttc atg cac ggc gcg ggc gcc 96
 Ala Tyr Gln Ala Gly Gly Pro Gly Pro Phe Met His Gly Ala Gly Ala
 20 25 30
 gcg tcc tcg cca gtc tac ctg ccc aca ccg cgg gtg ccc tcc tcc gtt 144
 Ala Ser Ser Pro Val Tyr Leu Pro Thr Pro Arg Val Pro Ser Ser Val
 35 40 45
 ctg ggc ctg tcc tac ctc cag ggc gga ggc gcg ggc tct gcg tcc gga 192
 Leu Gly Leu Ser Tyr Leu Gln Gly Gly Gly Ala Gly Ser Ala Ser Gly
 50 55 60
 ggc ccc tcg ggc ggc agc ccc ggt ggg gcc gcg tct ggt gcg ggg ccc 240
 Gly Pro Ser Gly Gly Ser Pro Gly Gly Ala Ala Ser Gly Ala Gly Pro
 65 70 75 80
 ggg acc cag cag ggc agc ccg gga tgg agc cag gcg gga gcg acc gga 288
 Gly Thr Gln Gln Gly Ser Pro Gly Trp Ser Gln Ala Gly Ala Thr Gly
 85 90 95
 gcc gct tac acc ccg ccg ccg gtg tcg ccg cgc ttc tcc ttc ccg ggg 336
 Ala Ala Tyr Thr Pro Pro Pro Val Ser Pro Arg Phe Ser Phe Pro Gly
 100 105 110
 acc acc ggg tcc ctg gcg gcg gcg gcg gcg gct gcc gcc gcc cgg gaa 384
 Thr Thr Gly Ser Leu Ala Ala Ala Ala Ala Ala Ala Ala Arg Glu
 115 120 125
 gct gcg gcc tac agc agt ggc ggc gga gcg gcg ggt gcg ggc ctg gcg 432

Ala Ala Ala Tyr Ser Ser Gly Gly Gly Ala Ala Gly Ala Gly Leu Ala	
130	135
140	
ggc cgc gag cag tac ggg cgc gcc ggc ttc gcg ggc tcc tac tcc agc	480
Gly Arg Glu Gln Tyr Gly Arg Ala Gly Phe Ala Gly Ser Tyr Ser Ser	
145	150
155	160
ccc tac ccg gct tac atg gcc gac gtg ggc gcg tcc tgg gcc gca gcc	528
Pro Tyr Pro Ala Tyr Met Ala Asp Val Gly Ala Ser Trp Ala Ala Ala	
165	170
175	
gcc gcc gcc tcc gcc ggc ccc ttc gac agc ccg gtc ctg cac agc ctg	576
Ala Ala Ala Ser Ala Gly Pro Phe Asp Ser Pro Val Leu His Ser Leu	
180	185
190	
ccc ggc cgg gcc aac ccg gcc gcc cga cac ccc aat ctc gat atg ttt	624
Pro Gly Arg Ala Asn Pro Ala Ala Arg His Pro Asn Leu Asp Met Phe	
195	200
205	
gac gac ttc tca gaa ggc aga gag tgt gtc aac tgt ggg gct atg tcc	672
Asp Asp Phe Ser Glu Gly Arg Glu Cys Val Asn Cys Gly Ala Met Ser	
210	215
220	
acc ccg ctc tgg agg cga gat ggg acg ggt cac tat ctg tgc aac gcc	720
Thr Pro Leu Trp Arg Arg Asp Gly Thr Gly His Tyr Leu Cys Asn Ala	
225	230
235	240
tgt ggc ctc tac cac aag atg aac ggc atc aac cgg ccg ctc atc aag	768
Cys Gly Leu Tyr His Lys Met Asn Gly Ile Asn Arg Pro Leu Ile Lys	
245	250
255	
cct cag cgc cgg ctg tcc gcc tcc cgc cga gtg ggc ctc tcc tgt gcc	816
Pro Gln Arg Arg Leu Ser Ala Ser Arg Arg Val Gly Leu Ser Cys Ala	
260	265
270	
aac tgc cag acc acc acc acc acc acg ctg tgg cgc cgc aat gcg gag ggc	864
Asn Cys Gln Thr Thr Thr Thr Thr Leu Trp Arg Arg Asn Ala Glu Gly	
275	280
285	
gag cct gtg tgc aat gcc tgc ggc ctc tac atg aag ctc cac ggg gtg	912
Glu Pro Val Cys Asn Ala Cys Gly Leu Tyr Met Lys Leu His Gly Val	
290	295
300	
ccc agg cct ctt gca atg cgg aaa gag ggg atc caa acc aga aaa cgg	960
Pro Arg Pro Leu Ala Met Arg Lys Glu Gly Ile Gln Thr Arg Lys Arg	
305	310
315	320
aag ccc aag aac ctg aat aaa tct aag aca cca gca gct cct tca ggc	1008

Lys	Pro	Lys	Asn	Leu	Asn	Lys	Ser	Lys	Thr	Pro	Ala	Ala	Pro	Ser	Gly		
				325					330					335			
agt	gag	agc	ctt	cct	ccc	gcc	agc	ggt	gct	tcc	agc	aac	tcc	agc	aac	1056	
Ser	Glu	Ser	Leu	Pro	Pro	Ala	Ser	Gly	Ala	Ser	Ser	Asn	Ser	Ser	Asn		
			340					345					350				
gcc	acc	acc	agc	agc	agc	gag	gag	atg	cgt	ccc	atc	aag	acg	gag	cct	1104	
Ala	Thr	Thr	Ser	Ser	Ser	Glu	Glu	Met	Arg	Pro	Ile	Lys	Thr	Glu	Pro		
			355					360					365				
ggc	ctg	tca	tct	cac	tac	ggg	cac	agc	agc	tcc	gtg	tcc	cag	acg	ttc	1152	
Gly	Leu	Ser	Ser	His	Tyr	Gly	His	Ser	Ser	Ser	Val	Ser	Gln	Thr	Phe		
	370					375					380						
tca	gtc	agt	gcg	atg	tct	ggc	cat	ggg	ccc	tcc	atc	cac	cct	gtc	ctc	1200	
Ser	Val	Ser	Ala	Met	Ser	Gly	His	Gly	Pro	Ser	Ile	His	Pro	Val	Leu		
385				390					395				400				
tcg	gcc	ctg	aag	ctc	tcc	cca	caa	ggc	tat	gcg	tct	ccc	gtc	agc	cag	1248	
Ser	Ala	Leu	Lys	Leu	Ser	Pro	Gln	Gly	Tyr	Ala	Ser	Pro	Val	Ser	Gln		
			405					410				415					
tct	cca	cag	acc	agc	tcc	aag	cag	gac	tct	tgg	aac	agt	ctg	gtc	ttg	1296	
Ser	Pro	Gln	Thr	Ser	Ser	Lys	Gln	Asp	Ser	Trp	Asn	Ser	Leu	Val	Leu		
			420					425				430					
gcc	gac	agt	cac	ggg	gac	ata	atc	act	gcg							1326	
Ala	Asp	Ser	His	Gly	Asp	Ile	Ile	Thr	Ala								
			435			440											

<210> 13
 <211> 507
 <212> PRT
 <213> Homo sapiens
 <400> 13

Met	Gly	Arg	Lys	Lys	Ile	Gln	Ile	Thr	Arg	Ile	Met	Asp	Glu	Arg	Asn		
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Arg	Gln	Val	Thr	Phe	Thr	Lys	Arg	Lys	Phe	Gly	Leu	Met	Lys	Lys	Ala		
			20					25					30				
Tyr	Glu	Leu	Ser	Val	Leu	Cys	Asp	Cys	Glu	Ile	Ala	Leu	Ile	Ile	Phe		
		35					40					45					
Asn	Ser	Ser	Asn	Lys	Leu	Phe	Gln	Tyr	Ala	Ser	Thr	Asp	Met	Asp	Lys		
	50					55					60						

Val	Leu	Leu	Lys	Tyr	Thr	Glu	Tyr	Asn	Glu	Pro	His	Glu	Ser	Arg	Thr	65	70	75	80
Asn	Ser	Asp	Ile	Val	Glu	Ala	Leu	Asn	Lys	Lys	Glu	His	Arg	Gly	Cys	85	90	95	
Asp	Ser	Pro	Asp	Pro	Asp	Thr	Ser	Tyr	Val	Leu	Thr	Pro	His	Thr	Glu	100	105	110	
Glu	Lys	Tyr	Lys	Lys	Ile	Asn	Glu	Glu	Phe	Asp	Asn	Met	Met	Arg	Asn	115	120	125	
His	Lys	Ile	Ala	Pro	Gly	Leu	Pro	Pro	Gln	Asn	Phe	Ser	Met	Ser	Val	130	135	140	
Thr	Val	Pro	Val	Thr	Ser	Pro	Asn	Ala	Leu	Ser	Tyr	Thr	Asn	Pro	Gly	145	150	155	160
Ser	Ser	Leu	Val	Ser	Pro	Ser	Leu	Ala	Ala	Ser	Ser	Thr	Leu	Thr	Asp	165	170	175	
Ser	Ser	Met	Leu	Ser	Pro	Pro	Gln	Thr	Thr	Leu	His	Arg	Asn	Val	Ser	180	185	190	
Pro	Gly	Ala	Pro	Gln	Arg	Pro	Pro	Ser	Thr	Gly	Asn	Ala	Gly	Gly	Met	195	200	205	
Leu	Ser	Thr	Thr	Asp	Leu	Thr	Val	Pro	Asn	Gly	Ala	Gly	Ser	Ser	Pro	210	215	220	
Val	Gly	Asn	Gly	Phe	Val	Asn	Ser	Arg	Ala	Ser	Pro	Asn	Leu	Ile	Gly	225	230	235	240
Ala	Thr	Gly	Ala	Asn	Ser	Leu	Gly	Lys	Val	Met	Pro	Thr	Lys	Ser	Pro	245	250	255	
Pro	Pro	Pro	Gly	Gly	Gly	Asn	Leu	Gly	Met	Asn	Ser	Arg	Lys	Pro	Asp	260	265	270	
Leu	Arg	Val	Val	Ile	Pro	Pro	Ser	Ser	Lys	Gly	Met	Met	Pro	Pro	Leu	275	280	285	
Ser	Glu	Glu	Glu	Glu	Leu	Glu	Leu	Asn	Thr	Gln	Arg	Ile	Ser	Ser	Ser	290	295	300	
Gln	Ala	Thr	Gln	Pro	Leu	Ala	Thr	Pro	Val	Val	Ser	Val	Thr	Thr	Pro	305	310	315	320
Ser	Leu	Pro	Pro	Gln	Gly	Leu	Val	Tyr	Ser	Ala	Met	Pro	Thr	Ala	Tyr	325	330	335	
Asn	Thr	Asp	Tyr	Ser	Leu	Thr	Ser	Ala	Asp	Leu	Ser	Ala	Leu	Gln	Gly	340	345	350	

Phe Asn Ser Pro Gly Met Leu Ser Leu Gly Gln Val Ser Ala Trp Gln
355 360 365
Gln His His Leu Gly Gln Ala Ala Leu Ser Ser Leu Val Ala Gly Gly
370 375 380
Gln Leu Ser Gln Gly Ser Asn Leu Ser Ile Asn Thr Asn Gln Asn Ile
385 390 395 400
Ser Ile Lys Ser Glu Pro Ile Ser Pro Pro Arg Asp Arg Met Thr Pro
405 410 415
Ser Gly Phe Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Pro Pro
420 425 430
Pro Pro Pro Gln Pro Gln Pro Gln Pro Pro Gln Pro Gln Pro Arg Gln
435 440 445
Glu Met Gly Arg Ser Pro Val Asp Ser Leu Ser Ser Ser Ser Ser Ser
450 455 460
Tyr Asp Gly Ser Asp Arg Glu Asp Pro Arg Gly Asp Phe His Ser Pro
465 470 475 480
Ile Val Leu Gly Arg Pro Pro Asn Thr Glu Asp Arg Glu Ser Pro Ser
485 490 495
Val Lys Arg Met Arg Met Asp Ala Trp Val Thr
500 505

<210> 14

<211> 1521

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(1524)

<400> 14

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Met Gly Arg Lys Lys Ile Gln Ile Thr Arg Ile Met Asp Glu Arg Asn
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cga cag gtc act ttt aca aag aga aag ttt gga tta atg aag aaa gcc 96
Arg Gln Val Thr Phe Thr Lys Arg Lys Phe Gly Leu Met Lys Lys Ala
20 25 30
tat gaa ctt agt gtg ctc tgt gac tgt gaa ata gca ctc atc att ttc 144
Tyr Glu Leu Ser Val Leu Cys Asp Cys Glu Ile Ala Leu Ile Ile Phe

35	40	45	
aac agc tct aac aaa ctg ttt caa tat gct agc act gat atg gac aaa	192		
Asn Ser Ser Asn Lys Leu Phe Gln Tyr Ala Ser Thr Asp Met Asp Lys			
50	55	60	
gtt ctt ctc aag tat aca gaa tat aat gaa cct cat gaa agc aga acc	240		
Val Leu Leu Lys Tyr Thr Glu Tyr Asn Glu Pro His Glu Ser Arg Thr			
65	70	75	80
aac tcg gat att gtt gag gct ctg aac aag aag gaa cac aga ggg tgc	288		
Asn Ser Asp Ile Val Glu Ala Leu Asn Lys Lys Glu His Arg Gly Cys			
85	90	95	
gac agc cca gac cct gat act tca tat gtg cta act cca cat aca gaa	336		
Asp Ser Pro Asp Pro Asp Thr Ser Tyr Val Leu Thr Pro His Thr Glu			
100	105	110	
gaa aaa tat aaa aaa att aat gag gaa ttt gat aat atg atg cgg aat	384		
Glu Lys Tyr Lys Lys Ile Asn Glu Glu Phe Asp Asn Met Met Arg Asn			
115	120	125	
cat aaa atc gca cct ggt ctg cca cct cag aac ttt tca atg tct gtc	432		
His Lys Ile Ala Pro Gly Leu Pro Pro Gln Asn Phe Ser Met Ser Val			
130	135	140	
aca gtt cca gtg acc agc ccc aat gct ttg tcc tac act aac cca ggg	480		
Thr Val Pro Val Thr Ser Pro Asn Ala Leu Ser Tyr Thr Asn Pro Gly			
145	150	155	160
agt tca ctg gtg tcc cca tct ttg gca gcc agc tca acg tta aca gat	528		
Ser Ser Leu Val Ser Pro Ser Leu Ala Ala Ser Ser Thr Leu Thr Asp			
165	170	175	
tca agc atg ctc tct cca cct caa acc aca tta cat aga aat gtg tct	576		
Ser Ser Met Leu Ser Pro Pro Gln Thr Thr Leu His Arg Asn Val Ser			
180	185	190	
cct gga gct cct cag aga cca cca agt act ggc aat gca ggt ggg atg	624		
Pro Gly Ala Pro Gln Arg Pro Pro Ser Thr Gly Asn Ala Gly Gly Met			
195	200	205	
ttg agc act aca gac ctc aca gtg cca aat gga gct gga agc agt cca	672		
Leu Ser Thr Thr Asp Leu Thr Val Pro Asn Gly Ala Gly Ser Ser Pro			
210	215	220	
gtg ggg aat gga ttt gta aac tca aga gct tct cca aat ttg att gga	720		
Val Gly Asn Gly Phe Val Asn Ser Arg Ala Ser Pro Asn Leu Ile Gly			

225	230	235	240	
gct act ggt gca aat agc tta ggc aaa gtc atg cct aca aag tct ccc				768
Ala Thr Gly Ala Asn Ser Leu Gly Lys Val Met Pro Thr Lys Ser Pro				
	245	250	255	
cct cca cca ggt ggt ggt aat ctt gga atg aac agt agg aaa cca gat				816
Pro Pro Pro Gly Gly Gly Asn Leu Gly Met Asn Ser Arg Lys Pro Asp				
	260	265	270	
ctt cga gtt gtc atc ccc cct tca agc aag ggc atg atg cct cca cta				864
Leu Arg Val Val Ile Pro Pro Ser Ser Lys Gly Met Met Pro Pro Leu				
	275	280	285	
tcg gag gaa gag gaa ttg gag ttg aac acc caa agg atc agt agt tct				912
Ser Glu Glu Glu Glu Leu Glu Leu Asn Thr Gln Arg Ile Ser Ser Ser				
	290	295	300	
caa gcc act caa cct ctt gct acc cca gtc gtg tct gtg aca acc cca				960
Gln Ala Thr Gln Pro Leu Ala Thr Pro Val Val Ser Val Thr Thr Pro				
	305	310	315	320
agc ttg cct ccg caa gga ctt gtg tac tca gca atg ccg act gcc tac				1008
Ser Leu Pro Pro Gln Gly Leu Val Tyr Ser Ala Met Pro Thr Ala Tyr				
	325	330	335	
aac act gat tat tca ctg acc agc gct gac ctg tca gcc ctt caa ggc				1056
Asn Thr Asp Tyr Ser Leu Thr Ser Ala Asp Leu Ser Ala Leu Gln Gly				
	340	345	350	
ttc aac tcg cca gga atg ctg tcg ctg gga cag gtg tcg gcc tgg cag				1104
Phe Asn Ser Pro Gly Met Leu Ser Leu Gly Gln Val Ser Ala Trp Gln				
	355	360	365	
cag cac cac cta gga caa gca gcc ctc agc tct ctt gtt gct gga ggg				1152
Gln His His Leu Gly Gln Ala Ala Leu Ser Ser Leu Val Ala Gly Gly				
	370	375	380	
cag tta tct cag ggt tcc aat tta tcc att aat acc aac caa aac atc				1200
Gln Leu Ser Gln Gly Ser Asn Leu Ser Ile Asn Thr Asn Gln Asn Ile				
	385	390	395	400
agc atc aag tcc gaa ccg att tca cct cct cgg gat cgt atg acc cca				1248
Ser Ile Lys Ser Glu Pro Ile Ser Pro Pro Arg Asp Arg Met Thr Pro				
	405	410	415	
tcg ggc ttc cag cag cag cag cag cag cag cag cag cag ccg ccg				1296
Ser Gly Phe Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Pro Pro				

cca cca ccg cag ccc cag cca caa ccc ccg cag ccc cag ccc cga cag	1344
Pro Pro Pro Gln Pro Gln Pro Gln Pro Pro Gln Pro Gln Pro Arg Gln	
435 440 445	
gaa atg ggg cgc tcc cct gtg gac agt ctg agc agc tct agt agc tcc	1392
Glu Met Gly Arg Ser Pro Val Asp Ser Leu Ser Ser Ser Ser Ser Ser	
450 455 460	
tat gat ggc agt gat cgg gag gat cca cgg ggc gac ttc cat tct cca	1440
Tyr Asp Gly Ser Asp Arg Glu Asp Pro Arg Gly Asp Phe His Ser Pro	
465 470 475 480	
att gtg ctt ggc cga ccc cca aac act gag gac aga gaa agc cct tct	1488
Ile Val Leu Gly Arg Pro Pro Asn Thr Glu Asp Arg Glu Ser Pro Ser	
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gta aag cga atg agg atg gac gcg tgg gtg acc	1521
Val Lys Arg Met Arg Met Asp Ala Trp Val Thr	
500 505	
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<400> 15	
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20 25 30	
Tyr Glu Leu Ser Val Leu Cys Asp Cys Glu Ile Ala Leu Ile Ile Phe	
35 40 45	
Asn Ser Ala Asn Arg Leu Phe Gln Tyr Ala Ser Thr Asp Met Asp Arg	
50 55 60	
Val Leu Leu Lys Tyr Thr Glu Tyr Ser Glu Pro His Glu Ser Arg Thr	
65 70 75 80	
Asn Thr Asp Ile Leu Glu Thr Leu Lys Arg Arg Gly Ile Gly Leu Asp	
85 90 95	
Gly Pro Glu Leu Glu Pro Asp Glu Gly Pro Glu Glu Pro Gly Glu Lys	
100 105 110	
Phe Arg Arg Leu Ala Gly Glu Gly Gly Asp Pro Ala Leu Pro Arg Pro	

115	120	125
Arg Leu Tyr Pro Ala Ala Pro Ala Met Pro Ser Pro Asp Val Val Tyr		
130	135	140
Gly Ala Leu Pro Pro Pro Gly Cys Asp Pro Ser Gly Leu Gly Glu Ala		
145	150	155
Leu Pro Ala Gln Ser Arg Pro Ser Pro Phe Arg Pro Ala Ala Pro Lys		160
	165	170
Ala Gly Pro Pro Gly Leu Val His Pro Leu Phe Ser Pro Ser His Leu		175
	180	185
Thr Ser Lys Thr Pro Pro Pro Leu Tyr Leu Pro Thr Glu Gly Arg Arg		190
	195	200
Ser Asp Leu Pro Gly Gly Leu Ala Gly Pro Arg Gly Gly Leu Asn Thr		205
	210	215
Ser Arg Ser Leu Tyr Ser Gly Leu Gln Asn Pro Cys Ser Thr Ala Thr		220
225	230	235
Pro Gly Pro Pro Leu Gly Ser Phe Pro Phe Leu Pro Gly Gly Pro Pro		240
	245	250
Val Gly Ala Glu Ala Trp Ala Arg Arg Val Pro Gln Pro Ala Ala Pro		255
	260	265
Pro Arg Arg Pro Pro Gln Ser Ala Ser Ser Leu Ser Ala Ser Leu Arg		270
	275	280
Pro Pro Gly Ala Pro Ala Thr Phe Leu Arg Pro Ser Pro Ile Pro Cys		285
	290	295
Ser Ser Pro Gly Pro Trp Gln Ser Leu Cys Gly Leu Gly Pro Pro Cys		300
305	310	315
Ala Gly Cys Pro Trp Pro Thr Ala Gly Pro Gly Arg Arg Ser Pro Gly		320
	325	330
Gly Thr Ser Pro Glu Arg Ser Pro Gly Thr Ala Arg Ala Arg Gly Asp		335
	340	345
Pro Thr Ser Leu Gln Ala Ser Ser Glu Lys Thr Gln Gln		350
	355	360

<210> 16

<211> 1095

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(1098)

<400> 16

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1 5 10 15	
cgg cag gtg acg ttc acc aag cgg aag ttc ggg ctg atg aag aag gcc	96
Arg Gln Val Thr Phe Thr Lys Arg Lys Phe Gly Leu Met Lys Lys Ala	
20 25 30	
tat gag ctg agc gtg ctc tgt gac tgt gag ata gcc ctc atc atc ttc	144
Tyr Glu Leu Ser Val Leu Cys Asp Cys Glu Ile Ala Leu Ile Ile Phe	
35 40 45	
aac agc gcc aac cgc ctc ttc cag tat gcc agc acg gac atg gac cgt	192
Asn Ser Ala Asn Arg Leu Phe Gln Tyr Ala Ser Thr Asp Met Asp Arg	
50 55 60	
gtg ctg ctg aag tac aca gag tac agc gag ccc cac gag agc cgc acc	240
Val Leu Leu Lys Tyr Thr Glu Tyr Ser Glu Pro His Glu Ser Arg Thr	
65 70 75 80	
aac act gac atc ctc gag acg ctg aag cgg agg ggc att ggc ctc gat	288
Asn Thr Asp Ile Leu Glu Thr Leu Lys Arg Arg Gly Ile Gly Leu Asp	
85 90 95	
ggg cca gag ctg gag ccg gat gaa ggg cct gag gag cca gga gag aag	336
Gly Pro Glu Leu Glu Pro Asp Glu Gly Pro Glu Glu Pro Gly Glu Lys	
100 105 110	
ttt cgg agg ctg gca ggc gaa ggg ggt gat ccg gcc ttg ccc cga ccc	384
Phe Arg Arg Leu Ala Gly Glu Gly Gly Asp Pro Ala Leu Pro Arg Pro	
115 120 125	
cgg ctg tat cct gca gct cct gct atg ccc agc cca gat gtg gta tac	432
Arg Leu Tyr Pro Ala Ala Pro Ala Met Pro Ser Pro Asp Val Val Tyr	
130 135 140	
ggg gcc tta ccg cca cca ggc tgt gac ccc agt ggg ctt ggg gaa gca	480
Gly Ala Leu Pro Pro Pro Gly Cys Asp Pro Ser Gly Leu Gly Glu Ala	
145 150 155 160	
ctg ccc gcc cag agc cgc cca tct ccc ttc cga cca gca gcc ccc aaa	528
Leu Pro Ala Gln Ser Arg Pro Ser Pro Phe Arg Pro Ala Ala Pro Lys	
165 170 175	

gcc ggg ccc cca ggc ctg gtg cac cct ctc ttc tca cca agc cac ctc	576
Ala Gly Pro Pro Gly Leu Val His Pro Leu Phe Ser Pro Ser His Leu	
180 185 190	
acc agc aag aca cca ccc cca ctg tac ctg ccg acg gaa ggg cgg agg	624
Thr Ser Lys Thr Pro Pro Pro Leu Tyr Leu Pro Thr Glu Gly Arg Arg	
195 200 205	
tca gac ctg cct ggt ggc ctg gct ggg ccc cga ggg gga cta aac acc	672
Ser Asp Leu Pro Gly Gly Leu Ala Gly Pro Arg Gly Gly Leu Asn Thr	
210 215 220	
tcc aga agc ctc tac agt ggc ctg cag aac ccc tgc tcc act gca act	720
Ser Arg Ser Leu Tyr Ser Gly Leu Gln Asn Pro Cys Ser Thr Ala Thr	
225 230 235 240	
ccc gga ccc cca ctg ggg agc ttc ccc ttc ctc ccc gga ggc ccc cca	768
Pro Gly Pro Pro Leu Gly Ser Phe Pro Phe Leu Pro Gly Gly Pro Pro	
245 250 255	
gtg ggg gcc gaa gcc tgg gcg agg agg gtc ccc caa ccc gcg gcg cct	816
Val Gly Ala Glu Ala Trp Ala Arg Arg Val Pro Gln Pro Ala Ala Pro	
260 265 270	
ccc cgc cga ccc ccc cag tca gca tca agt ctg agc gcc tct ctc cgg	864
Pro Arg Arg Pro Pro Gln Ser Ala Ser Ser Leu Ser Ala Ser Leu Arg	
275 280 285	
ccc ccg ggg gcc ccg gcg act ttc cta aga cct tcc cct atc cct tgc	912
Pro Pro Gly Ala Pro Ala Thr Phe Leu Arg Pro Ser Pro Ile Pro Cys	
290 295 300	
tcc tcg ccc ggt ccc tgg cag agc ctc tgc ggc ctg ggc ccg ccc tgc	960
Ser Ser Pro Gly Pro Trp Gln Ser Leu Cys Gly Leu Gly Pro Pro Cys	
305 310 315 320	
gcc ggc tgc cct tgg ccg acg gct ggc ccc ggt agg aga tca ccc ggt	1008
Ala Gly Cys Pro Trp Pro Thr Ala Gly Pro Gly Arg Arg Ser Pro Gly	
325 330 335	
ggc acc agc cca gag cgc tcg cca ggt acg gcg agg gca cgt ggg gac	1056
Gly Thr Ser Pro Glu Arg Ser Pro Gly Thr Ala Arg Ala Arg Gly Asp	
340 345 350	
ccc acc tcc ctc cag gcc tct tca gag aag acc caa cag	1095
Pro Thr Ser Leu Gln Ala Ser Ser Glu Lys Thr Gln Gln	
355 360 365	

<210> 17

<211> 465

<212> PRT

<213> Homo sapiens

<400> 17

Met Gly Arg Lys Lys Ile Gln Ile Thr Arg Ile Met Asp Glu Arg Asn
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20 25 30
Tyr Glu Leu Ser Val Leu Cys Asp Cys Glu Ile Ala Leu Ile Ile Phe
35 40 45
Asn Ser Thr Asn Lys Leu Phe Gln Tyr Ala Ser Thr Asp Met Asp Lys
50 55 60
Val Leu Leu Lys Tyr Thr Glu Tyr Asn Glu Pro His Glu Ser Arg Thr
65 70 75 80
Asn Ser Asp Ile Val Glu Thr Leu Arg Lys Lys Gly Leu Asn Gly Cys
85 90 95
Asp Ser Pro Asp Pro Asp Ala Asp Asp Ser Val Gly His Ser Pro Glu
100 105 110
Ser Glu Asp Lys Tyr Arg Lys Ile Asn Glu Asp Ile Asp Leu Met Ile
115 120 125
Ser Arg Gln Arg Leu Cys Ala Val Pro Pro Pro Asn Phe Glu Met Pro
130 135 140
Val Ser Ile Pro Val Ser Ser His Asn Ser Leu Val Tyr Ser Asn Pro
145 150 155 160
Val Ser Ser Leu Gly Asn Pro Asn Leu Leu Pro Leu Ala His Pro Ser
165 170 175
Leu Gln Arg Asn Ser Met Ser Pro Gly Val Thr His Arg Pro Pro Ser
180 185 190
Ala Gly Asn Thr Gly Gly Leu Met Gly Gly Asp Leu Thr Ser Gly Ala
195 200 205
Gly Thr Ser Ala Gly Asn Gly Tyr Gly Asn Pro Arg Asn Ser Pro Gly
210 215 220
Leu Leu Val Ser Pro Gly Asn Leu Asn Lys Asn Met Gln Ala Lys Ser
225 230 235 240
Pro Pro Pro Met Asn Leu Gly Met Asn Asn Arg Lys Pro Asp Leu Arg

atg ggg aga aaa aag att cag att acg agg att atg gat gaa cgt aac	48
Met Gly Arg Lys Lys Ile Gln Ile Thr Arg Ile Met Asp Glu Arg Asn	
1 5 10 15	
aga cag gtg aca ttt aca aag agg aaa ttt ggg ttg atg aag aag gct	96
Arg Gln Val Thr Phe Thr Lys Arg Lys Phe Gly Leu Met Lys Lys Ala	
20 25 30	
tat gag ctg agc gtg ctg tgt gac tgt gag att gcg ctg atc atc ttc	144
Tyr Glu Leu Ser Val Leu Cys Asp Cys Glu Ile Ala Leu Ile Ile Phe	
35 40 45	
aac agc acc aac aag ctg ttc cag tat gcc agc acc gac atg gac aaa	192
Asn Ser Thr Asn Lys Leu Phe Gln Tyr Ala Ser Thr Asp Met Asp Lys	
50 55 60	
gtg ctt ctc aag tac acg gag tac aac gag ccg cat gag agc cgg aca	240
Val Leu Leu Lys Tyr Thr Glu Tyr Asn Glu Pro His Glu Ser Arg Thr	
65 70 75 80	
aac tca gac atc gtg gag acg ttg aga aag aag ggc ctt aat ggc tgt	288
Asn Ser Asp Ile Val Glu Thr Leu Arg Lys Lys Gly Leu Asn Gly Cys	
85 90 95	
gac agc cca gac ccc gat gcg gac gat tcc gta ggt cac agc cct gag	336
Asp Ser Pro Asp Pro Asp Ala Asp Asp Ser Val Gly His Ser Pro Glu	
100 105 110	
tct gag gac aag tac agg aaa att aac gaa gat att gat cta atg atc	384
Ser Glu Asp Lys Tyr Arg Lys Ile Asn Glu Asp Ile Asp Leu Met Ile	
115 120 125	
agc agg caa aga ttg tgt gct gtt cca cct ccc aac ttc gag atg cca	432
Ser Arg Gln Arg Leu Cys Ala Val Pro Pro Pro Asn Phe Glu Met Pro	
130 135 140	
gtc tcc atc cca gtg tcc agc cac aac agt ttg gtg tac agc aac cct	480
Val Ser Ile Pro Val Ser Ser His Asn Ser Leu Val Tyr Ser Asn Pro	
145 150 155 160	
gtc agc tca ctg gga aac ccc aac cta ttg cca ctg gct cac cct tct	528
Val Ser Ser Leu Gly Asn Pro Asn Leu Leu Pro Leu Ala His Pro Ser	
165 170 175	
ctg cag agg aat agt atg tct cct ggt gta aca cat cga cct cca agt	576
Leu Gln Arg Asn Ser Met Ser Pro Gly Val Thr His Arg Pro Pro Ser	
180 185 190	

gca ggt aac aca ggt ggt ctg atg ggt gga gac ctc acg tct ggt gca	624
Ala Gly Asn Thr Gly Gly Leu Met Gly Gly Asp Leu Thr Ser Gly Ala	
195 200 205	
ggc acc agt gca ggg aac ggg tat ggc aat ccc cga aac tca cca ggt	672
Gly Thr Ser Ala Gly Asn Gly Tyr Gly Asn Pro Arg Asn Ser Pro Gly	
210 215 220	
ctg ctg gtc tca cct ggt aac ttg aac aag aat atg caa gca aaa tct	720
Leu Leu Val Ser Pro Gly Asn Leu Asn Lys Asn Met Gln Ala Lys Ser	
225 230 235 240	
cct ccc cca atg aat tta gga atg aat aac cgt aaa cca gat ctc cga	768
Pro Pro Pro Met Asn Leu Gly Met Asn Asn Arg Lys Pro Asp Leu Arg	
245 250 255	
gtt ctt att cca cca ggc agc aag aat acg atg cca tca gtg aat caa	816
Val Leu Ile Pro Pro Gly Ser Lys Asn Thr Met Pro Ser Val Asn Gln	
260 265 270	
agg ata aat aac tcc cag tcg gct cag tca ttg gct acc cca gtg gtt	864
Arg Ile Asn Asn Ser Gln Ser Ala Gln Ser Leu Ala Thr Pro Val Val	
275 280 285	
tcc gta gca act cct act tta cca gga caa gga atg gga gga tat cca	912
Ser Val Ala Thr Pro Thr Leu Pro Gly Gln Gly Met Gly Gly Tyr Pro	
290 295 300	
tca gcc att tca aca aca tat ggt acc gag tac tct ctg agt agt gca	960
Ser Ala Ile Ser Thr Thr Tyr Gly Thr Glu Tyr Ser Leu Ser Ser Ala	
305 310 315 320	
gac ctg tca tct ctg tct ggg ttt aac acc gcc agc gct ctt cac ctt	1008
Asp Leu Ser Ser Leu Ser Gly Phe Asn Thr Ala Ser Ala Leu His Leu	
325 330 335	
ggt tca gta act ggc tgg caa cag caa cac cta cat aac atg cca cca	1056
Gly Ser Val Thr Gly Trp Gln Gln Gln His Leu His Asn Met Pro Pro	
340 345 350	
tct gcc ctc agt cag ttg gga gct tgc act agc act cat tta tct cag	1104
Ser Ala Leu Ser Gln Leu Gly Ala Cys Thr Ser Thr His Leu Ser Gln	
355 360 365	
agt tca aat ctc tcc ctg cct tct act caa agc ctc aac atc aag tca	1152
Ser Ser Asn Leu Ser Leu Pro Ser Thr Gln Ser Leu Asn Ile Lys Ser	
370 375 380	

	100		105		110
Leu Glu Asp Lys Tyr Arg Arg Ala Ser Glu Glu Leu Asp Gly Leu Phe					
115		120		125	
Arg Arg Tyr Gly Ser Thr Val Pro Ala Pro Asn Phe Ala Met Pro Val					
130		135		140	
Thr Val Pro Val Ser Asn Gln Ser Ser Leu Gln Phe Ser Asn Pro Ser					
145		150		155	160
Gly Ser Leu Val Thr Pro Ser Leu Val Thr Ser Ser Leu Thr Asp Pro					
165		170		175	
Arg Leu Leu Ser Pro Gln Gln Pro Ala Leu Gln Arg Asn Ser Val Ser					
180		185		190	
Pro Gly Leu Pro Gln Arg Pro Ala Ser Ala Gly Ala Met Leu Gly Gly					
195		200		205	
Asp Leu Asn Ser Ala Asn Gly Ala Cys Pro Ser Pro Val Gly Asn Gly					
210		215		220	
Tyr Val Ser Ala Arg Ala Ser Pro Gly Leu Leu Pro Val Ala Asn Gly					
225		230		235	240
Asn Ser Leu Asn Lys Val Ile Pro Ala Lys Ser Pro Pro Pro Pro Thr					
245		250		255	
His Ser Thr Gln Leu Gly Ala Pro Ser Arg Lys Pro Asp Leu Arg Val					
260		265		270	
Ile Thr Ser Gln Ala Gly Lys Gly Leu Met His His Leu Thr Glu Asp					
275		280		285	
His Leu Asp Leu Asn Asn Ala Gln Arg Leu Gly Val Ser Gln Ser Thr					
290		295		300	
His Ser Leu Thr Thr Pro Val Val Ser Val Ala Thr Pro Ser Leu Leu					
305		310		315	320
Ser Gln Gly Leu Pro Phe Ser Ser Met Pro Thr Ala Tyr Asn Thr Asp					
325		330		335	
Tyr Gln Leu Thr Ser Ala Glu Leu Ser Ser Leu Pro Ala Phe Ser Ser					
340		345		350	
Pro Gly Gly Leu Ser Leu Gly Asn Val Thr Ala Trp Gln Gln Pro Gln					
355		360		365	
Gln Pro Gln Gln Pro Gln Gln Pro Gln Pro Pro Gln Gln Gln Pro Pro					
370		375		380	
Gln Pro Gln Gln Pro Gln Pro Gln Gln Pro Gln Gln Pro Gln Gln Pro					

385 390 395 400
 Pro Gln Gln Gln Ser His Leu Val Pro Val Ser Leu Ser Asn Leu Ile
 405 410 415
 Pro Gly Ser Pro Leu Pro His Val Gly Ala Ala Leu Thr Val Thr Thr
 420 425 430
 His Pro His Ile Ser Ile Lys Ser Glu Pro Val Ser Pro Ser Arg Glu
 435 440 445
 Arg Ser Pro Ala Pro Pro Pro Pro Ala Val Phe Pro Ala Ala Arg Pro
 450 455 460
 Glu Pro Gly Asp Gly Leu Ser Ser Pro Ala Gly Gly Ser Tyr Glu Thr
 465 470 475 480
 Gly Asp Arg Asp Asp Gly Arg Gly Asp Phe Gly Pro Thr Leu Gly Leu
 485 490 495
 Leu Arg Pro Ala Pro Glu Pro Glu Ala Glu Gly Ser Ala Val Lys Arg
 500 505 510
 Met Arg Leu Asp Thr Trp Thr Leu Lys
 515 520

<210> 20

<211> 1563

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(1566)

<400> 20

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 Met Gly Arg Lys Lys Ile Gln Ile Gln Arg Ile Thr Asp Glu Arg Asn
 1 5 10 15
 cga cag gtg act ttc acc aag cgg aag ttt ggc ctg atg aag aag gcg 96
 Arg Gln Val Thr Phe Thr Lys Arg Lys Phe Gly Leu Met Lys Lys Ala
 20 25 30
 tat gag ctg agc gtg cta tgt gac tgc gag atc gca ctc atc atc ttc 144
 Tyr Glu Leu Ser Val Leu Cys Asp Cys Glu Ile Ala Leu Ile Ile Phe
 35 40 45
 aac cac tcc aac aag ctg ttc cag tac gcc agc acc gac atg gac aag 192
 Asn His Ser Asn Lys Leu Phe Gln Tyr Ala Ser Thr Asp Met Asp Lys

50	55	60	
gtg ctg ctc aag tac acg gag tac aat gag cca cac gag agc cgc acc			240
Val Leu Leu Lys Tyr Thr Glu Tyr Asn Glu Pro His Glu Ser Arg Thr			
65	70	75	80
aac gcc gac atc atc gag acc ctg agg aag aag ggc ttc aat ggc tgc			288
Asn Ala Asp Ile Ile Glu Thr Leu Arg Lys Lys Gly Phe Asn Gly Cys			
85	90	95	
gac agc ccc gag ccc gac ggg gag gac tcg ctg gaa cag agc ccc ctg			336
Asp Ser Pro Glu Pro Asp Gly Glu Asp Ser Leu Glu Gln Ser Pro Leu			
100	105	110	
ctg gag gac aag tac cga cgc gcc agc gag gag ctc gac ggg ctc ttc			384
Leu Glu Asp Lys Tyr Arg Arg Ala Ser Glu Glu Leu Asp Gly Leu Phe			
115	120	125	
cgg cgc tat ggg tca act gtc ccg gcc ccc aac ttt gcc atg cct gtc			432
Arg Arg Tyr Gly Ser Thr Val Pro Ala Pro Asn Phe Ala Met Pro Val			
130	135	140	
acg gtg ccc gtg tcc aat cag agc tca ctg cag ttc agc aat ccc agc			480
Thr Val Pro Val Ser Asn Gln Ser Ser Leu Gln Phe Ser Asn Pro Ser			
145	150	155	160
ggc tcc ctg gtc acc cct tcc ctg gtg aca tca tcc ctc acg gac ccg			528
Gly Ser Leu Val Thr Pro Ser Leu Val Thr Ser Ser Leu Thr Asp Pro			
165	170	175	
cgg ctc ctg tcc ccc cag cag cca gca cta cag agg aac agt gtg tct			576
Arg Leu Leu Ser Pro Gln Gln Pro Ala Leu Gln Arg Asn Ser Val Ser			
180	185	190	
cct ggc ctg ccc cag cgg cca gct agt gcg ggg gcc atg ctg ggg ggt			624
Pro Gly Leu Pro Gln Arg Pro Ala Ser Ala Gly Ala Met Leu Gly Gly			
195	200	205	
gac ctg aac agt gct aac gga gcc tgc ccc agc cct gtt ggg aat ggc			672
Asp Leu Asn Ser Ala Asn Gly Ala Cys Pro Ser Pro Val Gly Asn Gly			
210	215	220	
tac gtc agt gct cgg gct tcc cct ggc ctc ctc cct gtg gcc aat ggc			720
Tyr Val Ser Ala Arg Ala Ser Pro Gly Leu Leu Pro Val Ala Asn Gly			
225	230	235	240
aac agc cta aac aag gtc atc cct gcc aag tct ccg ccc cca cct acc			768
Asn Ser Leu Asn Lys Val Ile Pro Ala Lys Ser Pro Pro Pro Pro Thr			

	245	250	255	
cac agc acc cag ctt gga gcc ccc agc cgc aag ccc gac ctg cga gtc				816
His Ser Thr Gln Leu Gly Ala Pro Ser Arg Lys Pro Asp Leu Arg Val				
	260	265	270	
atc act tcc cag gca gga aag ggg tta atg cat cac ttg act gag gac				864
Ile Thr Ser Gln Ala Gly Lys Gly Leu Met His His Leu Thr Glu Asp				
	275	280	285	
cat tta gat ctg aac aat gcc cag cgc ctt ggg gtc tcc cag tct act				912
His Leu Asp Leu Asn Asn Ala Gln Arg Leu Gly Val Ser Gln Ser Thr				
	290	295	300	
cat tcg ctc acc acc cca gtg gtt tct gtg gca acg ccg agt tta ctc				960
His Ser Leu Thr Thr Pro Val Val Ser Val Ala Thr Pro Ser Leu Leu				
	305	310	315	320
agc cag ggc ctc ccc ttc tct tcc atg ccc act gcc tac aac aca gat				1008
Ser Gln Gly Leu Pro Phe Ser Ser Met Pro Thr Ala Tyr Asn Thr Asp				
	325	330	335	
tac cag ttg acc agt gca gag ctc tcc tcc tta cca gcc ttt agt tca				1056
Tyr Gln Leu Thr Ser Ala Glu Leu Ser Ser Leu Pro Ala Phe Ser Ser				
	340	345	350	
cct ggg ggg ctg tcg cta ggc aat gtc act gcc tgg caa cag cca cag				1104
Pro Gly Gly Leu Ser Leu Gly Asn Val Thr Ala Trp Gln Gln Pro Gln				
	355	360	365	
cag ccc cag cag ccg cag cag cca cag cct cca cag cag cag cca ccg				1152
Gln Pro Gln Gln Pro Gln Gln Pro Gln Pro Pro Gln Gln Gln Pro Pro				
	370	375	380	
cag cca cag cag cca cag cca cag cag cct cag cag ccg caa cag cca				1200
Gln Pro Gln Gln Pro Gln Pro Gln Gln Pro Gln Gln Pro Gln Gln Pro				
	385	390	395	400
cct cag caa cag tcc cac ctg gtc cct gta tct ctc agc aac ctc atc				1248
Pro Gln Gln Gln Ser His Leu Val Pro Val Ser Leu Ser Asn Leu Ile				
	405	410	415	
ccg ggc agc ccc ctg ccc cac gtg ggt gct gcc ctc aca gtc acc acc				1296
Pro Gly Ser Pro Leu Pro His Val Gly Ala Ala Leu Thr Val Thr Thr				
	420	425	430	
cac ccc cac atc agc atc aag tca gaa ccg gtg tcc cca agc cgt gag				1344
His Pro His Ile Ser Ile Lys Ser Glu Pro Val Ser Pro Ser Arg Glu				

435	440	445	
cgc agc cct gcg cct ccc cct cca gct gtg ttc cca gct gcc cgc cct			1392
Arg Ser Pro Ala Pro Pro Pro Pro Ala Val Phe Pro Ala Ala Arg Pro			
450	455	460	
gag cct gcc gat ggt ctc agc agc cca gcc ggg gga tcc tat gag acg			1440
Glu Pro Gly Asp Gly Leu Ser Ser Pro Ala Gly Gly Ser Tyr Glu Thr			
465	470	475	480
gga gac cgg gat gac gga cgg ggg gac ttc ggg ccc aca ctg gcc ctg			1488
Gly Asp Arg Asp Asp Gly Arg Gly Asp Phe Gly Pro Thr Leu Gly Leu			
485	490	495	
ctg cgc cca gcc cca gag cct gag gct gag gcc tca gct gtg aag agg			1536
Leu Arg Pro Ala Pro Glu Pro Glu Ala Glu Gly Ser Ala Val Lys Arg			
500	505	510	
atg cgg ctt gat acc tgg aca tta aag			1563
Met Arg Leu Asp Thr Trp Thr Leu Lys			
515	520		
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<211> 217			
<212> PRT			
<213> Rattus norvegicus			
<400> 21			
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Gly Tyr Pro Phe Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala			
20 25 30			
Ser Arg Cys Ser His Glu Glu Asn Pro Tyr Phe His Gly Trp Leu Ile			
35 40 45			
Gly His Pro Glu Met Ser Pro Pro Asp Tyr Ser Met Ala Leu Ser Tyr			
50 55 60			
Ser Pro Glu Tyr Ala Ser Gly Ala Ala Gly Leu Asp His Ser His Tyr			
65 70 75 80			
Gly Gly Val Pro Pro Gly Ala Gly Pro Pro Gly Leu Gly Gly Pro Arg			
85 90 95			
Pro Val Lys Arg Arg Gly Thr Ala Asn Arg Lys Glu Arg Arg Arg Thr			
100 105 110			
Gln Ser Ile Asn Ser Ala Phe Ala Glu Leu Arg Glu Cys Ile Pro Asn			

115	120	125
Val Pro Ala Asp Thr Lys Leu Ser Lys Ile Lys Thr Leu Arg Leu Ala		
130	135	140
Thr Ser Tyr Ile Ala Tyr Leu Met Asp Leu Leu Ala Lys Asp Asp Gln		
145	150	155
Asn Gly Glu Ala Glu Ala Phe Lys Ala Glu Ile Lys Lys Thr Asp Val		
165	170	175
Lys Glu Glu Lys Arg Lys Lys Glu Leu Asn Glu Ile Leu Lys Ser Thr		
180	185	190
Val Ser Ser Asn Asp Lys Lys Thr Lys Gly Arg Thr Gly Trp Pro Gln		
195	200	205
His Val Trp Ala Leu Glu Leu Lys Gln		
210	215	

<210> 22

<211> 651

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<223> (1)..(654)

<400> 22

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Met Ser Leu Val Gly Gly Phe Pro His His Pro Val Val His His Glu	
1 5 10 15	
ggc tac ccg ttc gcc gca gcc gca gcc gcc gct gct gct gcc gcc gcc	96
Gly Tyr Pro Phe Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala	
20 25 30	
agc cgc tgc agt cac gag gag aac ccc tat ttc cac ggc tgg ctt att	144
Ser Arg Cys Ser His Glu Glu Asn Pro Tyr Phe His Gly Trp Leu Ile	
35 40 45	
ggc cac ccg gag atg tcg ccc ccc gac tac agc atg gcc ctg tcc tac	192
Gly His Pro Glu Met Ser Pro Pro Asp Tyr Ser Met Ala Leu Ser Tyr	
50 55 60	
agt ccc gag tac gcc agc ggt gcc gcg ggc ctg gac cac tcc cat tat	240
Ser Pro Glu Tyr Ala Ser Gly Ala Ala Gly Leu Asp His Ser His Tyr	
65 70 75 80	

ggg gga gtg ccg ccc ggt gcc ggg cct ccc ggc ctg ggg ggg ccg cgc	288
Gly Gly Val Pro Pro Gly Ala Gly Pro Pro Gly Leu Gly Gly Pro Arg	
85 90 95	
ccg gtg aag cgt cgg ggc acc gcc aac cgc aag gag cgg cgc agg act	336
Pro Val Lys Arg Arg Gly Thr Ala Asn Arg Lys Glu Arg Arg Arg Thr	
100 105 110	
cag agc atc aac agc gcc ttc gcc gag ctg cgc gag tgc atc ccc aac	384
Gln Ser Ile Asn Ser Ala Phe Ala Glu Leu Arg Glu Cys Ile Pro Asn	
115 120 125	
gtg ccc gcc gac acc aaa ctc tcc aaa atc aag act ctg cgc ctg gcc	432
Val Pro Ala Asp Thr Lys Leu Ser Lys Ile Lys Thr Leu Arg Leu Ala	
130 135 140	
acc agc tac atc gcc tac ctc atg gat ctg ctg gcc aag gac gac cag	480
Thr Ser Tyr Ile Ala Tyr Leu Met Asp Leu Leu Ala Lys Asp Asp Gln	
145 150 155 160	
aac gga gag gcg gag gcc ttc aag gcg gag atc aag aag acc gac gtg	528
Asn Gly Glu Ala Glu Ala Phe Lys Ala Glu Ile Lys Lys Thr Asp Val	
165 170 175	
aaa gag gag aag agg aag aaa gag ctg aat gaa atc ttg aaa agt aca	576
Lys Glu Glu Lys Arg Lys Lys Glu Leu Asn Glu Ile Leu Lys Ser Thr	
180 185 190	
gtg agc agc aac gac aag aaa acc aaa ggc cgg aca ggc tgg cca cag	624
Val Ser Ser Asn Asp Lys Lys Thr Lys Gly Arg Thr Gly Trp Pro Gln	
195 200 205	
cac gtc tgg gcc ctg gag ctc aag cag	651
His Val Trp Ala Leu Glu Leu Lys Gln	
210 215	
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<213> Homo sapiens	
<400> 23	
Met Asn Leu Val Gly Ser Tyr Ala His His His His His His Pro	
1 5 10 15	
His Pro Ala His Pro Met Leu His Glu Pro Phe Leu Phe Gly Pro Ala	
20 25 30	

His Pro Ala	His Pro Met Leu His Glu Pro Phe Leu Phe Gly Pro Ala	
20	25	30
tcg cgc tgt cat cag gaa agg ccc tac ttc cag agc tgg ctg ctg agc		144
Ser Arg Cys His Gln Glu Arg Pro Tyr Phe Gln Ser Trp Leu Leu Ser		
35	40	45
ccg gct gac gct gcc ccg gac ttc cct gcg ggc ggg ccg ccg ccc gcg		192
Pro Ala Asp Ala Ala Pro Asp Phe Pro Ala Gly Gly Pro Pro Pro Ala		
50	55	60
gcc gct gca gcc gcc acc gcc tat ggt cct gac gcc agg cct ggg cag		240
Ala Ala Ala Ala Ala Thr Ala Tyr Gly Pro Asp Ala Arg Pro Gly Gln		
65	70	75
agc ccc ggg cgg ctg gag gcg ctt ggc ggc cgt ctt ggc cgg cgg aaa		288
Ser Pro Gly Arg Leu Glu Ala Leu Gly Gly Arg Leu Gly Arg Arg Lys		
85	90	95
ggc tca gga ccc aag aag gag cgg aga cgc act gag agc att aac agc		336
Gly Ser Gly Pro Lys Lys Glu Arg Arg Arg Thr Glu Ser Ile Asn Ser		
100	105	110
gca ttc gcg gag ttg cgc gag tgc atc ccc aac gtg ccg gcc gac acc		384
Ala Phe Ala Glu Leu Arg Glu Cys Ile Pro Asn Val Pro Ala Asp Thr		
115	120	125
aag ctc tcc aag atc aag act ctg cgc cta gcc acc agc tac atc gcc		432
Lys Leu Ser Lys Ile Lys Thr Leu Arg Leu Ala Thr Ser Tyr Ile Ala		
130	135	140
tac ctg atg gac gtg ctg gcc aag gat gca cag tct ggc gat ccc gag		480
Tyr Leu Met Asp Val Leu Ala Lys Asp Ala Gln Ser Gly Asp Pro Glu		
145	150	155
gcc ttc aag gct gaa ctc aag aag gcg gat ggc ggc cgt gag agc aag		528
Ala Phe Lys Ala Glu Leu Lys Lys Ala Asp Gly Gly Arg Glu Ser Lys		
165	170	175
cgg aaa agg gag ctg cag cag cac gaa ggt ttt cct cct gcc ctg ggc		576
Arg Lys Arg Glu Leu Gln Gln His Glu Gly Phe Pro Pro Ala Leu Gly		
180	185	190
cca gtc gag aag agg att aaa gga cgc acc ggc tgg ccg cag caa gtc		624
Pro Val Glu Lys Arg Ile Lys Gly Arg Thr Gly Trp Pro Gln Gln Val		
195	200	205
tgg gcg ctg gag tta aac cag		645

Trp Ala Leu Glu Leu Asn Gln
 210 215
 <210> 25
 <211> 411
 <212> PRT
 <213> Homo sapiens
 <400> 25
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 1 5 10 15
 Glu Gly Val Trp Ser Pro Asp Ile Glu Gln Ser Phe Gln Glu Ala Leu
 20 25 30
 Ala Ile Tyr Pro Pro Cys Gly Arg Arg Lys Ile Ile Leu Ser Asp Glu
 35 40 45
 Gly Lys Met Tyr Gly Arg Asn Glu Leu Ile Ala Arg Tyr Ile Lys Leu
 50 55 60
 Arg Thr Gly Lys Thr Arg Thr Arg Lys Gln Val Ser Ser His Ile Gln
 65 70 75 80
 Val Leu Ala Arg Arg Lys Ser Arg Asp Phe His Ser Lys Leu Lys Asp
 85 90 95
 Gln Thr Ala Lys Asp Lys Ala Leu Gln His Met Ala Ala Met Ser Ser
 100 105 110
 Ala Gln Ile Val Ser Ala Thr Ala Ile His Asn Lys Leu Gly Leu Pro
 115 120 125
 Gly Ile Pro Arg Pro Thr Phe Pro Gly Ala Pro Gly Phe Trp Pro Gly
 130 135 140
 Met Ile Gln Thr Gly Gln Pro Gly Ser Ser Gln Asp Val Lys Pro Phe
 145 150 155 160
 Val Gln Gln Ala Tyr Pro Ile Gln Pro Ala Val Thr Ala Pro Ile Pro
 165 170 175
 Gly Phe Glu Pro Ala Ser Ala Pro Ala Pro Ser Val Pro Ala Trp Gln
 180 185 190
 Gly Arg Ser Ile Gly Thr Thr Lys Leu Arg Leu Val Glu Phe Ser Ala
 195 200 205
 Phe Leu Glu Gln Gln Arg Asp Pro Asp Ser Tyr Asn Lys His Leu Phe
 210 215 220
 Val His Ile Gly His Ala Asn His Ser Tyr Ser Asp Pro Leu Leu Glu

225		230		235		240									
Ser	Val	Asp	Ile	Arg	Gln	Ile	Tyr	Asp	Lys	Phe	Pro	Glu	Lys	Lys	Gly
		245						250					255		
Gly	Leu	Lys	Glu	Leu	Phe	Gly	Lys	Gly	Pro	Gln	Asn	Ala	Phe	Phe	Leu
		260						265					270		
Val	Lys	Phe	Trp	Ala	Asp	Leu	Asn	Cys	Asn	Ile	Gln	Asp	Asp	Ala	Gly
		275						280				285			
Ala	Phe	Tyr	Gly	Val	Thr	Ser	Gln	Tyr	Glu	Ser	Ser	Glu	Asn	Met	Thr
		290					295					300			
Val	Thr	Cys	Ser	Thr	Lys	Val	Cys	Ser	Phe	Gly	Lys	Gln	Val	Val	Glu
305					310					315				320	
Lys	Val	Glu	Thr	Glu	Tyr	Ala	Arg	Phe	Glu	Asn	Gly	Arg	Phe	Val	Tyr
				325					330				335		
Arg	Ile	Asn	Arg	Ser	Pro	Met	Cys	Glu	Tyr	Met	Ile	Asn	Phe	Ile	His
		340						345				350			
Lys	Leu	Lys	His	Leu	Pro	Glu	Lys	Tyr	Met	Met	Asn	Ser	Val	Leu	Glu
		355					360				365				
Asn	Phe	Thr	Ile	Leu	Leu	Val	Val	Thr	Asn	Arg	Asp	Thr	Gln	Glu	Thr
		370				375					380				
Leu	Leu	Cys	Met	Ala	Cys	Val	Phe	Glu	Val	Ser	Asn	Ser	Glu	His	Gly
385				390					395				400		
Ala	Gln	His	His	Ile	Tyr	Arg	Leu	Val	Lys	Asp					
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<210> 26

<211> 1233

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(1236)

<400> 26

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Met	Glu	Arg	Met	Ser	Asp	Ser	Ala	Asp	Lys	Pro	Ile	Asp	Asn	Asp	Ala	
1			5					10					15			
gaa	ggg	gtc	tgg	agc	ccc	gac	atc	gag	caa	agc	ttt	cag	gag	gcc	ctg	96
Glu	Gly	Val	Trp	Ser	Pro	Asp	Ile	Glu	Gln	Ser	Phe	Gln	Glu	Ala	Leu	

	20	25	30	
gct atc tat cca cca tgt ggg agg agg aaa atc atc tta tca gac gaa				144
Ala Ile Tyr Pro Pro Cys Gly Arg Arg Lys Ile Ile Leu Ser Asp Glu				
	35	40	45	
ggc aaa atg tat ggt agg aat gaa ttg ata gcc aga tac atc aaa ctc				192
Gly Lys Met Tyr Gly Arg Asn Glu Leu Ile Ala Arg Tyr Ile Lys Leu				
	50	55	60	
agg aca ggc aag acg agg acc aga aaa cag gtg tct agt cac att cag				240
Arg Thr Gly Lys Thr Arg Thr Arg Lys Gln Val Ser Ser His Ile Gln				
	65	70	75	80
gtt ctt gcc aga agg aaa tct cgt gat ttt cat tcc aag cta aag gat				288
Val Leu Ala Arg Arg Lys Ser Arg Asp Phe His Ser Lys Leu Lys Asp				
	85	90	95	
cag act gca aag gat aag gcc ctg cag cac atg gcg gcc atg tcc tca				336
Gln Thr Ala Lys Asp Lys Ala Leu Gln His Met Ala Ala Met Ser Ser				
	100	105	110	
gcc cag atc gtc tcg gcc act gcc att cat aac aag ctg ggg ctg cct				384
Ala Gln Ile Val Ser Ala Thr Ala Ile His Asn Lys Leu Gly Leu Pro				
	115	120	125	
ggg att cca cgc ccg acc ttc cca ggg gcg ccg ggg ttc tgg ccg gga				432
Gly Ile Pro Arg Pro Thr Phe Pro Gly Ala Pro Gly Phe Trp Pro Gly				
	130	135	140	
atg att caa aca ggg cag cca gga tcc tca caa gac gtc aag cct ttt				480
Met Ile Gln Thr Gly Gln Pro Gly Ser Ser Gln Asp Val Lys Pro Phe				
	145	150	155	160
gtg cag cag gcc tac ccc atc cag cca gcg gtc aca gcc ccc att cca				528
Val Gln Gln Ala Tyr Pro Ile Gln Pro Ala Val Thr Ala Pro Ile Pro				
	165	170	175	
ggg ttt gag cct gca tcg gcc cca gct ccc tca gtc cct gcc tgg caa				576
Gly Phe Glu Pro Ala Ser Ala Pro Ala Pro Ser Val Pro Ala Trp Gln				
	180	185	190	
ggt cgc tcc att ggc aca acc aag ctt cgc ctg gtg gaa ttt tca gct				624
Gly Arg Ser Ile Gly Thr Thr Lys Leu Arg Leu Val Glu Phe Ser Ala				
	195	200	205	
ttt ctc gag cag cag cga gac cca gac tcg tac aac aaa cac ctc ttc				672
Phe Leu Glu Gln Gln Arg Asp Pro Asp Ser Tyr Asn Lys His Leu Phe				

210	215	220	
gtg cac att ggg cat gcc aac cat tct tac agt gac cca ttg ctt gaa			720
Val His Ile Gly His Ala Asn His Ser Tyr Ser Asp Pro Leu Leu Glu			
225	230	235	240
tca gtg gac att cgt cag att tat gac aaa ttt cct gaa aag aaa ggt			768
Ser Val Asp Ile Arg Gln Ile Tyr Asp Lys Phe Pro Glu Lys Lys Gly			
	245	250	255
ggc tta aag gaa ctg ttt gga aag ggc cct caa aat gcc ttc ttc ctc			816
Gly Leu Lys Glu Leu Phe Gly Lys Gly Pro Gln Asn Ala Phe Phe Leu			
	260	265	270
gta aaa ttc tgg gct gat tta aac tgc aat att caa gat gat gct ggg			864
Val Lys Phe Trp Ala Asp Leu Asn Cys Asn Ile Gln Asp Asp Ala Gly			
	275	280	285
gct ttt tat ggt gta acc agt cag tac gag agt tct gaa aat atg aca			912
Ala Phe Tyr Gly Val Thr Ser Gln Tyr Glu Ser Ser Glu Asn Met Thr			
	290	295	300
gtc acc tgt tcc acc aaa gtt tgc tcc ttt ggg aag caa gta gta gaa			960
Val Thr Cys Ser Thr Lys Val Cys Ser Phe Gly Lys Gln Val Val Glu			
305	310	315	320
aaa gta gag acg gag tat gca agg ttt gag aat ggc cga ttt gta tac			1008
Lys Val Glu Thr Glu Tyr Ala Arg Phe Glu Asn Gly Arg Phe Val Tyr			
	325	330	335
cga ata aac cgc tcc cca atg tgt gaa tat atg atc aac ttc atc cac			1056
Arg Ile Asn Arg Ser Pro Met Cys Glu Tyr Met Ile Asn Phe Ile His			
	340	345	350
aag ctc aaa cac tta cca gag aaa tat atg atg aac agt gtt ttg gaa			1104
Lys Leu Lys His Leu Pro Glu Lys Tyr Met Met Asn Ser Val Leu Glu			
	355	360	365
aac ttc aca att tta ttg gtg gta aca aac agg gat aca caa gaa act			1152
Asn Phe Thr Ile Leu Leu Val Val Thr Asn Arg Asp Thr Gln Glu Thr			
	370	375	380
cta ctc tgc atg gcc tgt gtg ttt gaa gtt tca aat agt gaa cac gga			1200
Leu Leu Cys Met Ala Cys Val Phe Glu Val Ser Asn Ser Glu His Gly			
385	390	395	400
gca caa cat cat att tac agg ctt gta aag gac			1233
Ala Gln His His Ile Tyr Arg Leu Val Lys Asp			

405

410

<210> 27

<211> 427

<212> PRT

<213> Homo sapiens

<400> 27

Ile	Thr	Ser	Asn	Glu	Trp	Ser	Ser	Pro	Thr	Ser	Pro	Glu	Gly	Ser	Thr
1				5					10					15	
Ala	Ser	Gly	Gly	Ser	Gln	Ala	Leu	Asp	Lys	Pro	Ile	Asp	Asn	Asp	Ala
			20					25					30		
Glu	Gly	Val	Trp	Ser	Pro	Asp	Ile	Glu	Gln	Ser	Phe	Gln	Glu	Ala	Leu
		35					40					45			
Ala	Ile	Tyr	Pro	Pro	Cys	Gly	Arg	Arg	Lys	Ile	Ile	Leu	Ser	Asp	Glu
	50					55					60				
Gly	Lys	Met	Tyr	Gly	Arg	Asn	Glu	Leu	Ile	Ala	Arg	Tyr	Ile	Lys	Leu
65					70					75				80	
Arg	Thr	Gly	Lys	Thr	Arg	Thr	Arg	Lys	Gln	Val	Ser	Ser	His	Ile	Gln
			85						90					95	
Val	Leu	Ala	Arg	Arg	Lys	Ala	Arg	Glu	Ile	Gln	Ala	Lys	Leu	Lys	Asp
		100						105					110		
Gln	Ala	Ala	Lys	Asp	Lys	Ala	Leu	Gln	Ser	Met	Ala	Ala	Met	Ser	Ser
	115						120						125		
Ala	Gln	Ile	Ile	Ser	Ala	Thr	Ala	Phe	His	Ser	Ser	Met	Ala	Leu	Ala
	130					135					140				
Arg	Gly	Pro	Gly	Arg	Pro	Ala	Val	Ser	Gly	Phe	Trp	Gln	Gly	Ala	Leu
145					150					155				160	
Pro	Gly	Gln	Ala	Gly	Thr	Ser	His	Asp	Val	Lys	Pro	Phe	Ser	Gln	Gln
			165						170					175	
Thr	Tyr	Ala	Val	Gln	Pro	Pro	Leu	Pro	Leu	Pro	Gly	Phe	Glu	Ser	Pro
		180						185					190		
Ala	Gly	Pro	Ala	Pro	Ser	Pro	Ser	Ala	Pro	Pro	Ala	Pro	Pro	Trp	Gln
	195					200						205			
Gly	Arg	Ser	Val	Ala	Ser	Ser	Lys	Leu	Trp	Met	Leu	Glu	Phe	Ser	Ala
	210					215					220				
Phe	Leu	Glu	Gln	Gln	Gln	Asp	Pro	Asp	Thr	Tyr	Asn	Lys	His	Leu	Phe
225					230					235				240	

Val His Ile Gly Gln Ser Ser Pro Ser Tyr Ser Asp Pro Tyr Leu Glu
 245 250 255
 Ala Val Asp Ile Arg Gln Ile Tyr Asp Lys Phe Pro Glu Lys Lys Gly
 260 265 270
 Gly Leu Lys Asp Leu Phe Glu Arg Gly Pro Ser Asn Ala Phe Phe Leu
 275 280 285
 Val Lys Phe Trp Ala Asp Leu Asn Thr Asn Ile Glu Asp Glu Gly Ser
 290 295 300
 Ser Phe Tyr Gly Val Ser Ser Gln Tyr Glu Ser Pro Glu Asn Met Ile
 305 310 315 320
 Ile Thr Cys Ser Thr Lys Val Cys Ser Phe Gly Lys Gln Val Val Glu
 325 330 335
 Lys Val Glu Thr Glu Tyr Ala Arg Tyr Glu Asn Gly His Tyr Ser Tyr
 340 345 350
 Arg Ile His Arg Ser Pro Leu Cys Glu Tyr Met Ile Asn Phe Ile His
 355 360 365
 Lys Leu Lys His Leu Pro Glu Lys Tyr Met Met Asn Ser Val Leu Glu
 370 375 380
 Asn Phe Thr Ile Leu Gln Val Val Thr Asn Arg Asp Thr Gln Glu Thr
 385 390 395 400
 Leu Leu Cys Ile Ala Tyr Val Phe Glu Val Ser Ala Ser Glu His Gly
 405 410 415
 Ala Gln His His Ile Tyr Arg Leu Val Lys Glu
 420 425

<210> 28

<211> 1281

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(1284)

<400> 28

att acc tcc aac gag tgg agc tct ccc acc tcc cct gag ggg agc acc 48
 Ile Thr Ser Asn Glu Trp Ser Ser Pro Thr Ser Pro Glu Gly Ser Thr
 1 5 10 15
 gcc tct ggg ggc agt cag gca ctg gac aag ccc atc gac aat gac gca 96

Ala Ser Gly Gly Ser Gln Ala Leu Asp Lys Pro Ile Asp Asn Asp Ala	
20 25 30	
gag ggc gtg tgg agc ccg gat att gag cag agt ttc cag gag gcc ctc	144
Glu Gly Val Trp Ser Pro Asp Ile Glu Gln Ser Phe Gln Glu Ala Leu	
35 40 45	
gcc atc tac ccg ccc tgt ggc agg cgc aaa atc atc ctg tcg gac gag	192
Ala Ile Tyr Pro Pro Cys Gly Arg Arg Lys Ile Ile Leu Ser Asp Glu	
50 55 60	
ggc aag atg tat ggt cgg aac gag ctg att gcc cgc tac atc aag ctc	240
Gly Lys Met Tyr Gly Arg Asn Glu Leu Ile Ala Arg Tyr Ile Lys Leu	
65 70 75 80	
cgg aca ggg aag acc cgc acc agg aag cag gtc tcc agc cac atc cag	288
Arg Thr Gly Lys Thr Arg Thr Arg Lys Gln Val Ser Ser His Ile Gln	
85 90 95	
gtg ctg gct cgt cgc aaa gct cgc gag atc cag gcc aag cta aag gac	336
Val Leu Ala Arg Arg Lys Ala Arg Glu Ile Gln Ala Lys Leu Lys Asp	
100 105 110	
cag gca gct aag gac aag gcc ctg cag agc atg gct gcc atg tcg tct	384
Gln Ala Ala Lys Asp Lys Ala Leu Gln Ser Met Ala Ala Met Ser Ser	
115 120 125	
gca cag atc atc tcc gcc acg gcc ttc cac agt agc atg gcc ctc gcc	432
Ala Gln Ile Ile Ser Ala Thr Ala Phe His Ser Ser Met Ala Leu Ala	
130 135 140	
cgg ggc ccc ggc cgc cca gca gtc tca ggg ttt tgg caa gga gct ttg	480
Arg Gly Pro Gly Arg Pro Ala Val Ser Gly Phe Trp Gln Gly Ala Leu	
145 150 155 160	
cca ggc caa gcc gga acg tcc cat gat gtg aag cct ttc tct cag caa	528
Pro Gly Gln Ala Gly Thr Ser His Asp Val Lys Pro Phe Ser Gln Gln	
165 170 175	
acc tat gct gtc cag cct ccg ctg cct ctg cca ggg ttt gag tct cct	576
Thr Tyr Ala Val Gln Pro Pro Leu Pro Leu Pro Gly Phe Glu Ser Pro	
180 185 190	
gca ggg ccc gcc cca tcg ccc tct gcg ccc ccg gca ccc cca tgg cag	624
Ala Gly Pro Ala Pro Ser Pro Ser Ala Pro Pro Ala Pro Pro Trp Gln	
195 200 205	
ggc cgc agc gtg gcc agc tcc aag ctc tgg atg ttg gag ttc tct gcc	672

Gly Arg Ser Val Ala Ser Ser Lys Leu Trp Met Leu Glu Phe Ser Ala	
210	215
220	
ttc ctg gag cag cag cag gac ccg gac acg tac aac aag cac ctg ttc	720
Phe Leu Glu Gln Gln Gln Asp Pro Asp Thr Tyr Asn Lys His Leu Phe	
225	230
235	240
gtg cac att ggc cag tcc agc cca agc tac agc gac ccc tac ctc gaa	768
Val His Ile Gly Gln Ser Ser Pro Ser Tyr Ser Asp Pro Tyr Leu Glu	
245	250
255	
gcc gtg gac atc cgc caa atc tat gac aaa ttc ccg gag aaa aag ggt	816
Ala Val Asp Ile Arg Gln Ile Tyr Asp Lys Phe Pro Glu Lys Lys Gly	
260	265
270	
gga ctc aag gat ctc ttc gaa cgg gga ccc tcc aat gcc ttt ttt ctt	864
Gly Leu Lys Asp Leu Phe Glu Arg Gly Pro Ser Asn Ala Phe Phe Leu	
275	280
285	
gtg aag ttc tgg gca gac ctc aac acc aac atc gag gat gaa ggc agc	912
Val Lys Phe Trp Ala Asp Leu Asn Thr Asn Ile Glu Asp Glu Gly Ser	
290	295
300	
tcc ttc tat ggg gtc tcc agc cag tat gag agc ccc gag aac atg atc	960
Ser Phe Tyr Gly Val Ser Ser Gln Tyr Glu Ser Pro Glu Asn Met Ile	
305	310
315	320
atc acc tgc tcc acg aag gtc tgc tct ttc ggc aag cag gtg gtg gag	1008
Ile Thr Cys Ser Thr Lys Val Cys Ser Phe Gly Lys Gln Val Val Glu	
325	330
335	
aaa gtt gag aca gag tat gct cgc tat gag aat gga cac tac tct tac	1056
Lys Val Glu Thr Glu Tyr Ala Arg Tyr Glu Asn Gly His Tyr Ser Tyr	
340	345
350	
cgc atc cac cgg tcc ccg ctc tgt gag tac atg atc aac ttc atc cac	1104
Arg Ile His Arg Ser Pro Leu Cys Glu Tyr Met Ile Asn Phe Ile His	
355	360
365	
aag ctc aag cac ctc cct gag aag tac atg atg aac agc gtg ctg gag	1152
Lys Leu Lys His Leu Pro Glu Lys Tyr Met Met Asn Ser Val Leu Glu	
370	375
380	
aac ttc acc atc ctg cag gtg gtc acc aac aga gac aca cag gag acc	1200
Asn Phe Thr Ile Leu Gln Val Val Thr Asn Arg Asp Thr Gln Glu Thr	
385	390
395	400
ttg ctg tgc att gcc tat gtc ttt gag gtg tca gcc agt gag cac ggg	1248

Leu Leu Cys Ile Ala Tyr Val Phe Glu Val Ser Ala Ser Glu His Gly
 405 410 415

gct cag cac cac atc tac agg ctg gtg aaa gaa 1281

Ala Gln His His Ile Tyr Arg Leu Val Lys Glu
 420 425

<210> 29

<211> 435

<212> PRT

<213> Homo sapiens

<400> 29

Ile Ala Ser Asn Ser Trp Asn Ala Ser Ser Ser Pro Gly Glu Ala Arg
 1 5 10 15
 Glu Asp Gly Pro Glu Gly Leu Asp Lys Gly Leu Asp Asn Asp Ala Glu
 20 25 30
 Gly Val Trp Ser Pro Asp Ile Glu Gln Ser Phe Gln Glu Ala Leu Ala
 35 40 45
 Ile Tyr Pro Pro Cys Gly Arg Arg Lys Ile Ile Leu Ser Asp Glu Gly
 50 55 60
 Lys Met Tyr Gly Arg Asn Glu Leu Ile Ala Arg Tyr Ile Lys Leu Arg
 65 70 75 80
 Thr Gly Lys Thr Arg Thr Arg Lys Gln Val Ser Ser His Ile Gln Val
 85 90 95
 Leu Ala Arg Lys Lys Val Arg Glu Tyr Gln Val Gly Ile Lys Ala Met
 100 105 110
 Asn Leu Asp Gln Val Ser Lys Asp Lys Ala Leu Gln Ser Met Ala Ser
 115 120 125
 Met Ser Ser Ala Gln Ile Val Ser Ala Ser Val Leu Gln Asn Lys Phe
 130 135 140
 Ser Pro Pro Ser Pro Leu Pro Gln Ala Val Phe Ser Thr Ser Ser Arg
 145 150 155 160
 Phe Trp Ser Ser Pro Pro Leu Leu Gly Gln Gln Pro Gly Pro Ser Gln
 165 170 175
 Asp Ile Lys Pro Phe Ala Gln Pro Ala Tyr Pro Ile Gln Pro Pro Leu
 180 185 190
 Pro Pro Thr Leu Ser Ser Tyr Glu Pro Leu Ala Pro Leu Pro Ser Ala
 195 200 205

Ala	Ala	Ser	Val	Pro	Val	Trp	Gln	Asp	Arg	Thr	Ile	Ala	Ser	Ser	Arg
210						215					220				
Leu	Arg	Leu	Leu	Glu	Tyr	Ser	Ala	Phe	Met	Glu	Val	Gln	Arg	Asp	Pro
225				230						235					240
Asp	Thr	Tyr	Ser	Lys	His	Leu	Phe	Val	His	Ile	Gly	Gln	Thr	Asn	Pro
			245						250					255	
Ala	Phe	Ser	Asp	Pro	Pro	Leu	Glu	Ala	Val	Asp	Val	Arg	Gln	Ile	Tyr
			260					265					270		
Asp	Lys	Phe	Pro	Glu	Lys	Lys	Gly	Gly	Leu	Lys	Glu	Leu	Tyr	Glu	Lys
	275						280					285			
Gly	Pro	Pro	Asn	Ala	Phe	Phe	Leu	Val	Lys	Phe	Trp	Ala	Asp	Leu	Asn
	290					295					300				
Ser	Thr	Ile	Gln	Glu	Gly	Pro	Gly	Ala	Phe	Tyr	Gly	Val	Ser	Ser	Gln
305				310						315					320
Tyr	Ser	Ser	Ala	Asp	Ser	Met	Thr	Ile	Ser	Val	Ser	Thr	Lys	Val	Cys
			325						330					335	
Ser	Phe	Gly	Lys	Gln	Val	Val	Glu	Lys	Val	Glu	Thr	Glu	Tyr	Ala	Arg
			340					345					350		
Leu	Glu	Asn	Gly	Arg	Phe	Val	Tyr	Arg	Ile	His	Arg	Ser	Pro	Met	Cys
	355						360					365			
Glu	Tyr	Met	Ile	Asn	Phe	Ile	His	Lys	Leu	Lys	His	Leu	Pro	Glu	Lys
	370					375					380				
Tyr	Met	Met	Asn	Ser	Val	Leu	Glu	Asn	Phe	Thr	Ile	Leu	Gln	Val	Val
385				390						395					400
Thr	Ser	Arg	Asp	Ser	Gln	Glu	Thr	Leu	Leu	Val	Ile	Ala	Phe	Val	Phe
			405						410				415		
Glu	Val	Ser	Thr	Ser	Glu	His	Gly	Ala	Gln	His	His	Val	Tyr	Lys	Leu
			420					425					430		

Val Lys Asp

<210> 30

<211> 1305

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(1305)

<400> 30

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Ile Ala Ser Asn Ser Trp Asn Ala Ser Ser Ser Pro Gly Glu Ala Arg	
1 5 10 15	
gag gat ggg ccc gag ggc ctg gac aag ggg ctg gac aac gat gcg gag	96
Glu Asp Gly Pro Glu Gly Leu Asp Lys Gly Leu Asp Asn Asp Ala Glu	
20 25 30	
ggc gtg tgg agc ccg gac atc gag cag agc ttc cag gag gcc ctg gcc	144
Gly Val Trp Ser Pro Asp Ile Glu Gln Ser Phe Gln Glu Ala Leu Ala	
35 40 45	
atc tac ccg ccc tgc ggc cgg cgg aag atc atc ctg tca gac gag ggc	192
Ile Tyr Pro Pro Cys Gly Arg Arg Lys Ile Ile Leu Ser Asp Glu Gly	
50 55 60	
aag atg tac ggc cga aat gag ttg att gca cgc tat att aaa ctg agg	240
Lys Met Tyr Gly Arg Asn Glu Leu Ile Ala Arg Tyr Ile Lys Leu Arg	
65 70 75 80	
acg ggg aag act cgg acg aga aaa cag gtg tcc agc cac ata cag gtt	288
Thr Gly Lys Thr Arg Thr Arg Lys Gln Val Ser Ser His Ile Gln Val	
85 90 95	
cta gct cgg aag aag gtg cgg gag tac cag gtt ggc atc aag gcc atg	336
Leu Ala Arg Lys Lys Val Arg Glu Tyr Gln Val Gly Ile Lys Ala Met	
100 105 110	
aac ctg gac cag gtc tcc aag gac aaa gcc ctt cag agc atg gcg tcc	384
Asn Leu Asp Gln Val Ser Lys Asp Lys Ala Leu Gln Ser Met Ala Ser	
115 120 125	
atg tcc tct gcc cag atc gtc tct gcc agt gtc ctg cag aac aag ttc	432
Met Ser Ser Ala Gln Ile Val Ser Ala Ser Val Leu Gln Asn Lys Phe	
130 135 140	
agc cca cct tcc cct ctg ccc cag gcc gtc ttc tcc act tcc teg cgg	480
Ser Pro Pro Ser Pro Leu Pro Gln Ala Val Phe Ser Thr Ser Ser Arg	
145 150 155 160	
ttc tgg agc agc ccc cct ctc ctg gga cag cag cct gga ccc tct cag	528
Phe Trp Ser Ser Pro Pro Leu Leu Gly Gln Gln Pro Gly Pro Ser Gln	
165 170 175	
gac atc aag ccc ttt gca cag cca gcc tac ccc atc cag ccg ccc ctg	576
Asp Ile Lys Pro Phe Ala Gln Pro Ala Tyr Pro Ile Gln Pro Pro Leu	

	180	185	190	
ccg ccg acg ctc agc agt tat gag ccc ctg gcc ccg ctc ccc tca gct				624
Pro Pro Thr Leu Ser Ser Tyr Glu Pro Leu Ala Pro Leu Pro Ser Ala				
195	200	205		
gct gcc tct gtg cct gtg tgg cag gac cgt acc att gcc tcc tcc cgg				672
Ala Ala Ser Val Pro Val Trp Gln Asp Arg Thr Ile Ala Ser Ser Arg				
210	215	220		
ctg cgg ctc ctg gag tat tca gcc ttc atg gag gtg cag cga gac cct				720
Leu Arg Leu Leu Glu Tyr Ser Ala Phe Met Glu Val Gln Arg Asp Pro				
225	230	235	240	
gac acg tac agc aaa cac ctg ttt gtg cac atc ggc cag acg aac ccc				768
Asp Thr Tyr Ser Lys His Leu Phe Val His Ile Gly Gln Thr Asn Pro				
245	250	255		
gcc ttc tca gac cca ccc ctg gag gca gta gat gtg cgc cag atc tat				816
Ala Phe Ser Asp Pro Pro Leu Glu Ala Val Asp Val Arg Gln Ile Tyr				
260	265	270		
gac aaa ttc ccc gag aaa aag gga gga ttg aag gag ctc tat gag aag				864
Asp Lys Phe Pro Glu Lys Lys Gly Gly Leu Lys Glu Leu Tyr Glu Lys				
275	280	285		
ggg ccc cct aat gcc ttc ttc ctt gtc aag ttc tgg gcc gac ctc aac				912
Gly Pro Pro Asn Ala Phe Phe Leu Val Lys Phe Trp Ala Asp Leu Asn				
290	295	300		
agc acc atc cag gag ggc ccg gga gcc ttc tat ggg gtc agc tct cag				960
Ser Thr Ile Gln Glu Gly Pro Gly Ala Phe Tyr Gly Val Ser Ser Gln				
305	310	315	320	
tac agc tct gct gat agc atg acc atc agc gtc tcc acc aag gtg tgc				1008
Tyr Ser Ser Ala Asp Ser Met Thr Ile Ser Val Ser Thr Lys Val Cys				
325	330	335		
tcc ttt ggc aaa cag gtg gta gag aag gtg gag act gag tat gcc agg				1056
Ser Phe Gly Lys Gln Val Val Glu Lys Val Glu Thr Glu Tyr Ala Arg				
340	345	350		
ctg gag aac ggg cgc ttt gtg tac cgt atc cac cgc tcg ccc atg tgc				1104
Leu Glu Asn Gly Arg Phe Val Tyr Arg Ile His Arg Ser Pro Met Cys				
355	360	365		
gag tac atg atc aac ttc atc cac aag ctg aag cac ctg ccc gag aag				1152
Glu Tyr Met Ile Asn Phe Ile His Lys Leu Lys His Leu Pro Glu Lys				

370	375	380	
tac atg atg aac agc gtg ctg gag aac ttc acc atc ctg cag gtg gtc			1200
Tyr Met Met Asn Ser Val Leu Glu Asn Phe Thr Ile Leu Gln Val Val			
385	390	395	400
acg agc cgg gac tcc cag gag acc ttg ctt gtc att gct ttt gtc ttc			1248
Thr Ser Arg Asp Ser Gln Glu Thr Leu Leu Val Ile Ala Phe Val Phe			
405	410	415	
gaa gtc tcc acc agt gag cac ggg gcc cag cac cat gtc tac aag ctc			1296
Glu Val Ser Thr Ser Glu His Gly Ala Gln His His Val Tyr Lys Leu			
420	425	430	
gtc aaa gac			1305
Val Lys Asp			
435			
<210> 31			
<211> 1132			
<212> PRT			
<213> Homo sapiens			
<400> 31			
Met Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg Ser			
1 5 10 15			
His Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu Gly			
20 25 30			
Pro Gln Gly Trp Arg Leu Val Gln Arg Gly Asp Pro Ala Ala Phe Arg			
35 40 45			
Ala Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg Pro			
50 55 60			
Pro Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu			
65 70 75 80			
Val Ala Arg Val Leu Gln Arg Leu Cys Glu Arg Gly Ala Lys Asn Val			
85 90 95			
Leu Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro Pro			
100 105 110			
Glu Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr			
115 120 125			
Asp Ala Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Arg Arg Val			
130 135 140			

Gly	Asp	Asp	Val	Leu	Val	His	Leu	Leu	Ala	Arg	Cys	Ala	Leu	Phe	Val	145	150	155	160
Leu	Val	Ala	Pro	Ser	Cys	Ala	Tyr	Gln	Val	Cys	Gly	Pro	Pro	Leu	Tyr	165	170	175	
Gln	Leu	Gly	Ala	Ala	Thr	Gln	Ala	Arg	Pro	Pro	Pro	His	Ala	Ser	Gly	180	185	190	
Pro	Arg	Arg	Arg	Leu	Gly	Cys	Glu	Arg	Ala	Trp	Asn	His	Ser	Val	Arg	195	200	205	
Glu	Ala	Gly	Val	Pro	Leu	Gly	Leu	Pro	Ala	Pro	Gly	Ala	Arg	Arg	Arg	210	215	220	
Gly	Gly	Ser	Ala	Ser	Arg	Ser	Leu	Pro	Leu	Pro	Lys	Arg	Pro	Arg	Arg	225	230	235	240
Gly	Ala	Ala	Pro	Glu	Pro	Glu	Arg	Thr	Pro	Val	Gly	Gln	Gly	Ser	Trp	245	250	255	
Ala	His	Pro	Gly	Arg	Thr	Arg	Gly	Pro	Ser	Asp	Arg	Gly	Phe	Cys	Val	260	265	270	
Val	Ser	Pro	Ala	Arg	Pro	Ala	Glu	Glu	Ala	Thr	Ser	Leu	Glu	Gly	Ala	275	280	285	
Leu	Ser	Gly	Thr	Arg	His	Ser	His	Pro	Ser	Val	Gly	Arg	Gln	His	His	290	295	300	
Ala	Gly	Pro	Pro	Ser	Thr	Ser	Arg	Pro	Pro	Arg	Pro	Trp	Asp	Thr	Pro	305	310	315	320
Cys	Pro	Pro	Val	Tyr	Ala	Glu	Thr	Lys	His	Phe	Leu	Tyr	Ser	Ser	Gly	325	330	335	
Asp	Lys	Glu	Gln	Leu	Arg	Pro	Ser	Phe	Leu	Leu	Ser	Ser	Leu	Arg	Pro	340	345	350	
Ser	Leu	Thr	Gly	Ala	Arg	Arg	Leu	Val	Glu	Thr	Ile	Phe	Leu	Gly	Ser	355	360	365	
Arg	Pro	Trp	Met	Pro	Gly	Thr	Pro	Arg	Arg	Leu	Pro	Arg	Leu	Pro	Gln	370	375	380	
Arg	Tyr	Trp	Gln	Met	Arg	Pro	Leu	Phe	Leu	Glu	Leu	Leu	Gly	Asn	His	385	390	395	400
Ala	Gln	Cys	Pro	Tyr	Gly	Val	Leu	Leu	Lys	Thr	His	Cys	Pro	Leu	Arg	405	410	415	
Ala	Ala	Val	Thr	Pro	Ala	Ala	Gly	Val	Cys	Ala	Arg	Glu	Lys	Pro	Gln	420	425	430	

Gly	Ser	Val	Ala	Ala	Pro	Glu	Glu	Glu	Asp	Thr	Asp	Pro	Arg	Arg	Leu			
		435						440					445					
Val	Gln	Leu	Leu	Arg	Gln	His	Ser	Ser	Pro	Trp	Gln	Val	Tyr	Gly	Phe			
		450				455						460						
Val	Arg	Ala	Cys	Leu	Arg	Arg	Leu	Val	Pro	Pro	Gly	Leu	Trp	Gly	Ser			
465					470					475					480			
Arg	His	Asn	Glu	Arg	Arg	Phe	Leu	Arg	Asn	Thr	Lys	Lys	Phe	Ile	Ser			
				485					490						495			
Leu	Gly	Lys	His	Ala	Lys	Leu	Ser	Leu	Gln	Glu	Leu	Thr	Trp	Lys	Met			
			500					505					510					
Ser	Val	Arg	Asp	Cys	Ala	Trp	Leu	Arg	Arg	Ser	Pro	Gly	Val	Gly	Cys			
		515					520					525						
Val	Pro	Ala	Ala	Glu	His	Arg	Leu	Arg	Glu	Glu	Ile	Leu	Ala	Lys	Phe			
		530				535					540							
Leu	His	Trp	Leu	Met	Ser	Val	Tyr	Val	Val	Glu	Leu	Leu	Arg	Ser	Phe			
545					550					555					560			
Phe	Tyr	Val	Thr	Glu	Thr	Thr	Phe	Gln	Lys	Asn	Arg	Leu	Phe	Phe	Tyr			
				565					570						575			
Arg	Lys	Ser	Val	Trp	Ser	Lys	Leu	Gln	Ser	Ile	Gly	Ile	Arg	Gln	His			
			580					585					590					
Leu	Lys	Arg	Val	Gln	Leu	Arg	Glu	Leu	Ser	Glu	Ala	Glu	Val	Arg	Gln			
		595					600					605						
His	Arg	Glu	Ala	Arg	Pro	Ala	Leu	Leu	Thr	Ser	Arg	Leu	Arg	Phe	Ile			
		610				615					620							
Pro	Lys	Pro	Asp	Gly	Leu	Arg	Pro	Ile	Val	Asn	Met	Asp	Tyr	Val	Val			
625					630					635					640			
Gly	Ala	Arg	Thr	Phe	Arg	Arg	Glu	Lys	Arg	Ala	Glu	Arg	Leu	Thr	Ser			
				645					650						655			
Arg	Val	Lys	Ala	Leu	Phe	Ser	Val	Leu	Asn	Tyr	Glu	Arg	Ala	Arg	Arg			
			660					665					670					
Pro	Gly	Leu	Leu	Gly	Ala	Ser	Val	Leu	Gly	Leu	Asp	Asp	Ile	His	Arg			
		675					680					685						
Ala	Trp	Arg	Thr	Phe	Val	Leu	Arg	Val	Arg	Ala	Gln	Asp	Pro	Pro	Pro			
		690				695					700							
Glu	Leu	Tyr	Phe	Val	Lys	Val	Asp	Val	Thr	Gly	Ala	Tyr	Asp	Thr	Ile			
705					710					715					720			

Pro	Gln	Asp	Arg	Leu	Thr	Glu	Val	Ile	Ala	Ser	Ile	Ile	Lys	Pro	Gln	725	730	735
Asn	Thr	Tyr	Cys	Val	Arg	Arg	Tyr	Ala	Val	Val	Gln	Lys	Ala	Ala	His	740	745	750
Gly	His	Val	Arg	Lys	Ala	Phe	Lys	Ser	His	Val	Ser	Thr	Leu	Thr	Asp	755	760	765
Leu	Gln	Pro	Tyr	Met	Arg	Gln	Phe	Val	Ala	His	Leu	Gln	Glu	Thr	Ser	770	775	780
Pro	Leu	Arg	Asp	Ala	Val	Val	Ile	Glu	Gln	Ser	Ser	Ser	Leu	Asn	Glu	785	790	795
Ala	Ser	Ser	Gly	Leu	Phe	Asp	Val	Phe	Leu	Arg	Phe	Met	Cys	His	His	805	810	815
Ala	Val	Arg	Ile	Arg	Gly	Lys	Ser	Tyr	Val	Gln	Cys	Gln	Gly	Ile	Pro	820	825	830
Gln	Gly	Ser	Ile	Leu	Ser	Thr	Leu	Leu	Cys	Ser	Leu	Cys	Tyr	Gly	Asp	835	840	845
Met	Glu	Asn	Lys	Leu	Phe	Ala	Gly	Ile	Arg	Arg	Asp	Gly	Leu	Leu	Leu	850	855	860
Arg	Leu	Val	Asp	Asp	Phe	Leu	Leu	Val	Thr	Pro	His	Leu	Thr	His	Ala	865	870	875
Lys	Thr	Phe	Leu	Arg	Thr	Leu	Val	Arg	Gly	Val	Pro	Glu	Tyr	Gly	Cys	885	890	895
Val	Val	Asn	Leu	Arg	Lys	Thr	Val	Val	Asn	Phe	Pro	Val	Glu	Asp	Glu	900	905	910
Ala	Leu	Gly	Gly	Thr	Ala	Phe	Val	Gln	Met	Pro	Ala	His	Gly	Leu	Phe	915	920	925
Pro	Trp	Cys	Gly	Leu	Leu	Leu	Asp	Thr	Arg	Thr	Leu	Glu	Val	Gln	Ser	930	935	940
Asp	Tyr	Ser	Ser	Tyr	Ala	Arg	Thr	Ser	Ile	Arg	Ala	Ser	Leu	Thr	Phe	945	950	955
Asn	Arg	Gly	Phe	Lys	Ala	Gly	Arg	Asn	Met	Arg	Arg	Lys	Leu	Phe	Gly	965	970	975
Val	Leu	Arg	Leu	Lys	Cys	His	Ser	Leu	Phe	Leu	Asp	Leu	Gln	Val	Asn	980	985	990
Ser	Leu	Gln	Thr	Val	Cys	Thr	Asn	Ile	Tyr	Lys	Ile	Leu	Leu	Leu	Gln	995	1000	1005

Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
 1010 1015 1020
 Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
 1025 1030 1035 1040
 Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
 1045 1050 1055
 Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
 1060 1065 1070
 Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
 1075 1080 1085
 Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
 1090 1095 1100
 Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
 1105 1110 1115 1120
 Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
 1125 1130

<210> 32

<211> 3396

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(3399)

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Met Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg Ser	
1 5 10 15	
cac tac cgc gag gtg ctg ccg ctg gcc acg ttc gtg cgg cgc ctg ggg	96
His Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu Gly	
20 25 30	
ccc cag gcc tgg cgg ctg gtg cag cgc ggg gac ccg gcg gct ttc cgc	144
Pro Gln Gly Trp Arg Leu Val Gln Arg Gly Asp Pro Ala Ala Phe Arg	
35 40 45	
gcg ctg gtg gcc cag tgc ctg gtg tgc gtg ccc tgg gac gca cgg ccg	192
Ala Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg Pro	
50 55 60	

ccc ccc gcc gcc ccc tcc ttc cgc cag gtg tcc tgc ctg aag gag ctg	240
Pro Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu	
65 70 75 80	
gtg gcc cga gtg ctg cag agg ctg tgc gag cgc ggc gcg aag aac gtg	288
Val Ala Arg Val Leu Gln Arg Leu Cys Glu Arg Gly Ala Lys Asn Val	
85 90 95	
ctg gcc ttc ggc ttc gcg ctg ctg gac ggg gcc cgc ggg ggc ccc ccc	336
Leu Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro Pro	
100 105 110	
gag gcc ttc acc acc agc gtg cgc agc tac ctg ccc aac acg gtg acc	384
Glu Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr	
115 120 125	
gac gca ctg cgg ggg agc ggg gcg tgg ggg ctg ctg ctg cgc cgc gtg	432
Asp Ala Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Leu Arg Arg Val	
130 135 140	
ggc gac gac gtg ctg gtt cac ctg ctg gca cgc tgc gcg ctc ttt gtg	480
Gly Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Phe Val	
145 150 155 160	
ctg gtg gct ccc agc tgc gcc tac cag gtg tgc ggg ccg ccg ctg tac	528
Leu Val Ala Pro Ser Cys Ala Tyr Gln Val Cys Gly Pro Pro Leu Tyr	
165 170 175	
cag ctc ggc gct gcc act cag gcc cgg ccc ccg cca cac gct agt gga	576
Gln Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser Gly	
180 185 190	
ccc cga agg cgt ctg gga tgc gaa cgg gcc tgg aac cat agc gtc agg	624
Pro Arg Arg Arg Leu Gly Cys Glu Arg Ala Trp Asn His Ser Val Arg	
195 200 205	
gag gcc ggg gtc ccc ctg ggc ctg cca gcc ccg ggt gcg agg agg cgc	672
Glu Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg Arg	
210 215 220	
ggg ggc agt gcc agc cga agt ctg ccg ttg ccc aag agg ccc agg cgt	720
Gly Gly Ser Ala Ser Arg Ser Leu Pro Leu Pro Lys Arg Pro Arg Arg	
225 230 235 240	
ggc gct gcc cct gag ccg gag cgg acg ccc gtt ggg cag ggg tcc tgg	768
Gly Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser Trp	
245 250 255	

gcc cac ccg ggc agg acg cgt gga ccg agt gac cgt ggt ttc tgt gtg	816
Ala His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys Val	
260 265 270	
gtg tca cct gcc aga ccc gcc gaa gaa gcc acc tct ttg gag ggt gcg	864
Val Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly Ala	
275 280 285	
ctc tct ggc acg cgc cac tcc cac cca tcc gtg ggc cgc cag cac cac	912
Leu Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His His	
290 295 300	
gcg ggc ccc cca tcc aca tcg cgg cca cca cgt ccc tgg gac acg cct	960
Ala Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr Pro	
305 310 315 320	
tgt ccc ccg gtg tac gcc gag acc aag cac ttc ctc tac tcc tca ggc	1008
Cys Pro Pro Val Tyr Ala Glu Thr Lys His Phe Leu Tyr Ser Ser Gly	
325 330 335	
gac aag gag cag ctg cgg ccc tcc ttc cta ctc agc tct ctg agg ccc	1056
Asp Lys Glu Gln Leu Arg Pro Ser Phe Leu Leu Ser Ser Leu Arg Pro	
340 345 350	
agc ctg act ggc gct cgg agg ctc gtg gag acc atc ttt ctg ggt tcc	1104
Ser Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly Ser	
355 360 365	
agg ccc tgg atg cca ggg act ccc cgc agg ttg ccc cgc ctg ccc cag	1152
Arg Pro Trp Met Pro Gly Thr Pro Arg Arg Leu Pro Arg Leu Pro Gln	
370 375 380	
cgc tac tgg caa atg cgg ccc ctg ttt ctg gag ctg ctt ggg aac cac	1200
Arg Tyr Trp Gln Met Arg Pro Leu Phe Leu Glu Leu Leu Gly Asn His	
385 390 395 400	
gcg cag tgc ccc tac ggg gtg ctc ctc aag acg cac tgc ccg ctg cga	1248
Ala Gln Cys Pro Tyr Gly Val Leu Leu Lys Thr His Cys Pro Leu Arg	
405 410 415	
gct gcg gtc acc cca gca gcc ggt gtc tgt gcc cgg gag aag ccc cag	1296
Ala Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro Gln	
420 425 430	
ggc tct gtg gcg gcc ccc gag gag gag gac aca gac ccc cgt cgc ctg	1344
Gly Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg Leu	
435 440 445	

gtg cag ctg ctc cgc cag cac agc agc ccc tgg cag gtg tac ggc ttc	1392
Val Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly Phe	
450 455 460	
gtg cgg gcc tgc ctg cgc cgg ctg gtg ccc cca ggc ctc tgg ggc tcc	1440
Val Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly Ser	
465 470 475 480	
agg cac aac gaa cgc cgc ttc ctc agg aac acc aag aag ttc atc tcc	1488
Arg His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile Ser	
485 490 495	
ctg ggg aag cat gcc aag ctc tcg ctg cag gag ctg acg tgg aag atg	1536
Leu Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys Met	
500 505 510	
agc gtg cgg gac tgc gct tgg ctg cgc agg agc cca ggg gtt ggc tgt	1584
Ser Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly Cys	
515 520 525	
gtt ccg gcc gca gag cac cgt ctg cgt gag gag atc ctg gcc aag ttc	1632
Val Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys Phe	
530 535 540	
ctg cac tgg ctg atg agt gtg tac gtc gtc gag ctg ctc agg tct ttc	1680
Leu His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser Phe	
545 550 555 560	
ttt tat gtc acg gag acc acg ttt caa aag aac agg ctc ttt ttc tac	1728
Phe Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr	
565 570 575	
cgg aag agt gtc tgg agc aag ttg caa agc att gga atc aga cag cac	1776
Arg Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln His	
580 585 590	
ttg aag agg gtg cag ctg cgg gag ctg tcg gaa gca gag gtc agg cag	1824
Leu Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg Gln	
595 600 605	
cat cgg gaa gcc agg ccc gcc ctg ctg acg tcc aga ctc cgc ttc atc	1872
His Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe Ile	
610 615 620	
ccc aag cct gac ggg ctg cgg ccg att gtg aac atg gac tac gtc gtg	1920
Pro Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val Val	
625 630 635 640	

gga gcc aga acg ttc cgc aga gaa aag agg gcc gag cgt ctc acc tcg	1968
Gly Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser	
645 650 655	
agg gtg aag gca ctg ttc agc gtg ctc aac tac gag cgg gcg cgg cgc	2016
Arg Val Lys Ala Leu Phe Ser Val Leu Asn Tyr Glu Arg Ala Arg Arg	
660 665 670	
ccc ggc ctc ctg ggc gcc tct gtg ctg ggc ctg gac gat atc cac agg	2064
Pro Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His Arg	
675 680 685	
gcc tgg cgc acc ttc gtg ctg cgt gtg cgg gcc cag gac ccg ccg cct	2112
Ala Trp Arg Thr Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro Pro	
690 695 700	
gag ctg tac ttt gtc aag gtg gat gtg acg ggc gcg tac gac acc atc	2160
Glu Leu Tyr Phe Val Lys Val Asp Val Thr Gly Ala Tyr Asp Thr Ile	
705 710 715 720	
ccc cag gac agg ctc acg gag gtc atc gcc agc atc atc aaa ccc cag	2208
Pro Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro Gln	
725 730 735	
aac acg tac tgc gtg cgt cgg tat gcc gtg gtc cag aag gcc gcc cat	2256
Asn Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Gln Lys Ala Ala His	
740 745 750	
ggg cac gtc cgc aag gcc ttc aag agc cac gtc tct acc ttg aca gac	2304
Gly His Val Arg Lys Ala Phe Lys Ser His Val Ser Thr Leu Thr Asp	
755 760 765	
ctc cag ccg tac atg cga cag ttc gtg gct cac ctg cag gag acc agc	2352
Leu Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr Ser	
770 775 780	
ccg ctg agg gat gcc gtc gtc atc gag cag agc tcc tcc ctg aat gag	2400
Pro Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu	
785 790 795 800	
gcc agc agt ggc ctc ttc gac gtc ttc cta cgc ttc atg tgc cac cac	2448
Ala Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His	
805 810 815	
gcc gtg cgc atc agg ggc aag tcc tac gtc cag tgc cag ggg atc ccg	2496
Ala Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro	
820 825 830	

cag ggc tcc atc ctc tcc acg ctg ctc tgc agc ctg tgc tac ggc gac	2544
Gln Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp	
835 840 845	
atg gag aac aag ctg ttt gcg ggg att cgg cgg gac ggg ctg ctc ctg	2592
Met Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu	
850 855 860	
cgt ttg gtg gat gat ttc ttg ttg gtg aca cct cac ctc acc cac gcg	2640
Arg Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala	
865 870 875 880	
aaa acc ttc ctc agg acc ctg gtc cga ggt gtc cct gag tat ggc tgc	2688
Lys Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys	
885 890 895	
gtg gtg aac ttg cgg aag aca gtg gtg aac ttc cct gta gaa gac gag	2736
Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu	
900 905 910	
gcc ctg ggt ggc acg gct ttt gtt cag atg ccg gcc cac ggc cta ttc	2784
Ala Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe	
915 920 925	
ccc tgg tgc ggc ctg ctg ctg gat acc cgg acc ctg gag gtg cag agc	2832
Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser	
930 935 940	
gac tac tcc agc tat gcc cgg acc tcc atc aga gcc agt ctc acc ttc	2880
Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe	
945 950 955 960	
aac cgc ggc ttc aag gct ggg agg aac atg cgt cgc aaa ctc ttt ggg	2928
Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly	
965 970 975	
gtc ttg cgg ctg aag tgt cac agc ctg ttt ctg gat ttg cag gtg aac	2976
Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn	
980 985 990	
agc ctc cag acg gtg tgc acc aac atc tac aag atc ctc ctg ctg cag	3024
Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln	
995 1000 1005	
gcg tac agg ttt cac gca tgt gtg ctg cag ctc cca ttt cat cag caa	3072
Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln	
1010 1015 1020	

<223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 34
 aagagggcag atctatcgga 20
 <210> 35
 <211> 20
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 <220>
 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 35
 atggatctcc tgaaggtgct 20
 <210> 36
 <211> 20
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 36
 aagagggcag atctatcgga 20
 <210> 37
 <211> 23
 <212> DNA
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 37
 ggaagagtga gcggccatca agg 23
 <210> 38
 <211> 22
 <212> DNA
 <213> Artificial Sequence
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<223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 38
 ctgctggaga ggatttcct cg 22
 <210> 39
 <211> 24
 <212> DNA
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 39
 gccaacacca acctgtccaa gttc 24
 <210> 40
 <211> 24
 <212> DNA
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 40
 tgcaaaggct ccaggtctga gggc 24
 <210> 41
 <211> 19
 <212> DNA
 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 41
 ctctctctcc tcaggacaa 19
 <210> 42
 <211> 22
 <212> DNA
 <213> Artificial Sequence
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<223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 42
 tggagcaaaa cagaatggct gg 22
 <210> 43
 <211> 24
 <212> DNA
 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 43
 ctgagatgtc tctctctctc ttag 24
 <210> 44
 <211> 20
 <212> DNA
 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 44
 acaatgactg atgagagatg 20
 <210> 45
 <211> 18
 <212> DNA
 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 45
 cagacctgaa ggagacct 18
 <210> 46
 <211> 18
 <212> DNA
 <213> Artificial Sequence
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<223> Description of Artificial Sequence: artificially synthesized primer
sequence

<400> 46

gtcagcgtaa acagttgc

18

<210> 47

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificially synthesized primer
sequence

<400> 47

gccaaagaagc ggatagaagg

20

<210> 48

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificially synthesized primer
sequence

<400> 48

ctgtggttca gggctcagtc

20

<210> 49

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificially synthesized primer
sequence

<400> 49

cagtggagct ggacaaagcc

20

<210> 50

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 50
 tagcgacggt tctggaacca 20
 <210> 51
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 <212> DNA
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 51
 ctgtcatctc actatgggca 20
 <210> 52
 <211> 20
 <212> DNA
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 52
 ccaagtccga gcaggaattt 20
 <210> 53
 <211> 20
 <212> DNA
 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 53
 aagacgtcaa gccctttgtg 20
 <210> 54
 <211> 20
 <212> DNA
 <213> Artificial Sequence
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<223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 54
 aaaggagcac actttggtgg 20
 <210> 55
 <211> 20
 <212> DNA
 <213> Artificial Sequence
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 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 55
 agcaagaata cgatgccatc 20
 <210> 56
 <211> 20
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223>Description of Artificial Sequence: artificially
 synthesized primer sequence
 <400> 56
 gaaggggtgg tggtaggtc 20
 <210> 57
 <211> 20
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: artificially synthesized primer
 sequence
 <400> 57
 tgggaatggc tatgtcagtg 20
 <210> 58
 <211> 20
 <212> DNA
 <213> Artificial Sequence
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<223> Description of Artificial Sequence: artificially synthesized primer sequence

<400> 58

ctggtaatct gtgtttagg

20

<210> 59

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificially synthesized primer sequence

<400> 59

caagggcctc tccaaacttg

20

<210> 60

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: artificially synthesized primer sequence

<400> 60

gccccagaga cagcattcca

20

<210> 61

<211> 268

<212> PRT

<213> Homo sapiens

<400> 61

Met Ala Gln Pro Leu Cys Pro Pro Leu Ser Glu Ser Trp Met Leu Ser

1

5

10

15

Ala Ala Trp Gly Pro Thr Arg Arg Pro Pro Pro Ser Asp Lys Asp Cys

20

25

30

Gly Arg Ser Leu Val Ser Ser Pro Asp Ser Trp Gly Ser Thr Pro Ala

35

40

45

Asp Ser Pro Val Ala Ser Pro Ala Arg Pro Gly Thr Leu Arg Asp Pro
 50 55 60

Arg Ala Pro Ser Val Gly Arg Arg Gly Ala Arg Ser Ser Arg Leu Gly
 65 70 75 80

Ser Gly Gln Arg Gln Ser Ala Ser Glu Arg Glu Lys Leu Arg Met Arg
 85 90 95

Thr Leu Ala Arg Ala Leu His Glu Leu Arg Arg Phe Leu Pro Pro Ser
 100 105 110

Val Ala Pro Ala Gly Gln Ser Leu Thr Lys Ile Glu Thr Leu Arg Leu
 115 120 125

Ala Ile Arg Tyr Ile Gly His Leu Ser Ala Val Leu Gly Leu Ser Glu
 130 135 140

Glu Ser Leu Gln Arg Arg Cys Arg Gln Arg Gly Asp Ala Gly Ser Pro
 145 150 155 160

Arg Gly Cys Pro Leu Cys Pro Asp Asp Cys Pro Ala Gln Met Gln Thr
 165 170 175

Arg Thr Gln Ala Glu Gly Gln Gly Gln Gly Arg Gly Leu Gly Leu Val
 180 185 190

Ser Ala Val Arg Ala Gly Ala Ser Trp Gly Ser Pro Pro Ala Cys Pro
 195 200 205

Gly Ala Arg Ala Ala Pro Glu Pro Arg Asp Pro Pro Ala Leu Phe Ala
 210 215 220

Glu Ala Ala Cys Pro Glu Gly Gln Ala Met Glu Pro Ser Pro Pro Ser
 225 230 235 240

Pro Leu Leu Pro Gly Asp Val Leu Ala Leu Leu Glu Thr Trp Met Pro
245 250 255

Leu Ser Pro Leu Glu Trp Leu Pro Glu Glu Pro Lys
260 265

<210> 62

<211> 804

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(807)

<400> 62

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Met Ala Gln Pro Leu Cys Pro Pro Leu Ser Glu Ser Trp Met Leu Ser
1 5 10 15

gcg gcc tgg ggc cca act cgg cgg ccg ccg ccc tcc gac aag gac tgc 96
Ala Ala Trp Gly Pro Thr Arg Arg Pro Pro Pro Ser Asp Lys Asp Cys
20 25 30

ggc cgc tcc ctc gtc tcg tcc cca gac tca tgg ggc agc acc cca gcc 144
Gly Arg Ser Leu Val Ser Ser Pro Asp Ser Trp Gly Ser Thr Pro Ala
35 40 45

gac agc ccc gtg gcg agc ccc gcg cgg cca ggc acc ctc cgg gac ccc 192
Asp Ser Pro Val Ala Ser Pro Ala Arg Pro Gly Thr Leu Arg Asp Pro
50 55 60

cgc gcc ccc tcc gta ggt agg cgc ggc gcg cgc agc agc cgc ctg ggc 240
Arg Ala Pro Ser Val Gly Arg Arg Gly Ala Arg Ser Ser Arg Leu Gly
65 70 75 80

agc ggg cag agg cag agc gcc agt gag cgg gag aaa ctg cgc atg cgc 288
Ser Gly Gln Arg Gln Ser Ala Ser Glu Arg Glu Lys Leu Arg Met Arg
85 90 95

acg ctg gcc cgc gcc ctg cac gag ctg cgc cgc ttt cta ccg ccg tcc	336
Thr Leu Ala Arg Ala Leu His Glu Leu Arg Arg Phe Leu Pro Pro Ser	
100 105 110	
gtg gcg ccc gcg ggc cag agc ctg acc aag atc gag acg ctg cgc ctg	384
Val Ala Pro Ala Gly Gln Ser Leu Thr Lys Ile Glu Thr Leu Arg Leu	
115 120 125	
gct atc cgc tat atc ggc cac ctg tgc gcc gtg cta ggc ctc agc gag	432
Ala Ile Arg Tyr Ile Gly His Leu Ser Ala Val Leu Gly Leu Ser Glu	
130 135 140	
gag agt ctc cag cgc cgg tgc cgg cag cgc ggt gac gcg ggg tcc cct	480
Glu Ser Leu Gln Arg Arg Cys Arg Gln Arg Gly Asp Ala Gly Ser Pro	
145 150 155 160	
cgg ggc tgc ccg ctg tgc ccc gac gac tgc ccc gcg cag atg cag aca	528
Arg Gly Cys Pro Leu Cys Pro Asp Asp Cys Pro Ala Gln Met Gln Thr	
165 170 175	
cgg acg cag gct gag ggg cag ggg cag ggg cgc ggg ctg ggc ctg gta	576
Arg Thr Gln Ala Glu Gly Gln Gly Gln Gly Arg Gly Leu Gly Leu Val	
180 185 190	
tcc gcc gtc cgc gcc ggg gcg tcc tgg gga tcc ccg cct gcc tgc ccc	624
Ser Ala Val Arg Ala Gly Ala Ser Trp Gly Ser Pro Pro Ala Cys Pro	
195 200 205	
gga gcc cga gct gca ccc gag ccg cgc gac ccg cct gcg ctg ttc gcc	672
Gly Ala Arg Ala Ala Pro Glu Pro Arg Asp Pro Pro Ala Leu Phe Ala	
210 215 220	
gag gcg gcg tgc cct gaa ggg cag gcg atg gag cca agc cca ccg tcc	720
Glu Ala Ala Cys Pro Glu Gly Gln Ala Met Glu Pro Ser Pro Pro Ser	
225 230 235 240	

ccg ctc ctt ccg ggc gac gtg ctg gct ctg ttg gag acc tgg atg ccc 768
 Pro Leu Leu Pro Gly Asp Val Leu Ala Leu Leu Glu Thr Trp Met Pro
 245 250 255

ctc tcg cct ctg gag tgg ctg cct gag gag ccc aag 804
 Leu Ser Pro Leu Glu Trp Leu Pro Glu Glu Pro Lys
 260 265

<210> 63

<211> 215

<212> PRT

<213> Homo sapiens

<400> 63

Met Gly Ser Pro Arg Ser Ala Leu Ser Cys Leu Leu Leu His Leu Leu
 1 5 10 15

Val Leu Cys Leu Gln Ala Gln Val Thr Val Gln Ser Ser Pro Asn Phe
 20 25 30

Thr Gln His Val Arg Glu Gln Ser Leu Val Thr Asp Gln Leu Ser Arg
 35 40 45

Arg Leu Ile Arg Thr Tyr Gln Leu Tyr Ser Arg Thr Ser Gly Lys His
 50 55 60

Val Gln Val Leu Ala Asn Lys Arg Ile Asn Ala Met Ala Glu Asp Gly
 65 70 75 80

Asp Pro Phe Ala Lys Leu Ile Val Glu Thr Asp Thr Phe Gly Ser Arg
 85 90 95

Val Arg Val Arg Gly Ala Glu Thr Gly Leu Tyr Ile Cys Met Asn Lys
 100 105 110

Lys Gly Lys Leu Ile Ala Lys Ser Asn Gly Lys Gly Lys Asp Cys Val
 115 120 125

Phe Thr Glu Ile Val Leu Glu Asn Asn Tyr Thr Ala Leu Gln Asn Ala
 130 135 140

Lys Tyr Glu Gly Trp Tyr Met Ala Phe Thr Arg Lys Gly Arg Pro Arg
 145 150 155 160

Lys Gly Ser Lys Thr Arg Gln His Gln Arg Glu Val His Phe Met Lys
 165 170 175

Arg Leu Pro Arg Gly His His Thr Thr Glu Gln Ser Leu Arg Phe Glu
 180 185 190

Phe Leu Asn Tyr Pro Pro Phe Thr Arg Ser Leu Arg Gly Ser Gln Arg
 195 200 205

Thr Trp Ala Pro Glu Pro Arg
 210

<210> 64

<211> 645

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<223> (1)..(648)

<400> 64

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 Met Gly Ser Pro Arg Ser Ala Leu Ser Cys Leu Leu Leu His Leu Leu
 1 5 10 15

gtc ctc tgc ctc caa gcc cag gta act gtt cag tcc tca cct aat ttt 96
 Val Leu Cys Leu Gln Ala Gln Val Thr Val Gln Ser Ser Pro Asn Phe
 20 25 30

aca cag cat gtg agg gag cag agc ctg gtg acg gat cag ctc agc cgc 144

Thr	Gln	His	Val	Arg	Glu	Gln	Ser	Leu	Val	Thr	Asp	Gln	Leu	Ser	Arg	
	35						40					45				
cgc	ctc	atc	cgg	acc	tac	caa	ctc	tac	agc	cgc	acc	agc	ggg	aag	cac	192
Arg	Leu	Ile	Arg	Thr	Tyr	Gln	Leu	Tyr	Ser	Arg	Thr	Ser	Gly	Lys	His	
	50					55					60					
gtg	cag	gtc	ctg	gcc	aac	aag	cgc	atc	aac	gcc	atg	gca	gag	gac	ggc	240
Val	Gln	Val	Leu	Ala	Asn	Lys	Arg	Ile	Asn	Ala	Met	Ala	Glu	Asp	Gly	
	65				70					75					80	
gac	ccc	ttc	gca	aag	ctc	atc	gtg	gag	acg	gac	acc	ttt	gga	agc	aga	288
Asp	Pro	Phe	Ala	Lys	Leu	Ile	Val	Glu	Thr	Asp	Thr	Phe	Gly	Ser	Arg	
				85					90					95		
gtt	cga	gtc	cga	gga	gcc	gag	acg	ggc	ctc	tac	atc	tgc	atg	aac	aag	336
Val	Arg	Val	Arg	Gly	Ala	Glu	Thr	Gly	Leu	Tyr	Ile	Cys	Met	Asn	Lys	
			100					105					110			
aag	ggg	aag	ctg	atc	gcc	aag	agc	aac	ggc	aaa	ggc	aag	gac	tgc	gtc	384
Lys	Gly	Lys	Leu	Ile	Ala	Lys	Ser	Asn	Gly	Lys	Gly	Lys	Asp	Cys	Val	
	115						120					125				
ttc	acg	gag	att	gtg	ctg	gag	aac	aac	tac	aca	gcg	ctg	cag	aat	gcc	432
Phe	Thr	Glu	Ile	Val	Leu	Glu	Asn	Asn	Tyr	Thr	Ala	Leu	Gln	Asn	Ala	
	130					135					140					
aag	tac	gag	ggc	tgg	tac	atg	gcc	ttc	acc	cgc	aag	ggc	cgg	ccc	cgc	480
Lys	Tyr	Glu	Gly	Trp	Tyr	Met	Ala	Phe	Thr	Arg	Lys	Gly	Arg	Pro	Arg	
	145				150					155				160		
aag	ggc	tcc	aag	acg	cgg	cag	cac	cag	cgt	gag	gtc	cac	ttc	atg	aag	528
Lys	Gly	Ser	Lys	Thr	Arg	Gln	His	Gln	Arg	Glu	Val	His	Phe	Met	Lys	
			165					170					175			
cgg	ctg	ccc	cgg	ggc	cac	cac	acc	acc	gag	cag	agc	ctg	cgc	ttc	gag	576

Arg Leu Pro Arg Gly His His Thr Thr Glu Gln Ser Leu Arg Phe Glu
180 185 190

ttc ctc aac tac ccg ccc ttc acg cgc agc ctg cgc ggc agc cag agg 624
Phe Leu Asn Tyr Pro Pro Phe Thr Arg Ser Leu Arg Gly Ser Gln Arg
195 200 205

act tgg gcc ccg gaa ccc cga 645
Thr Trp Ala Pro Glu Pro Arg
210 215

<210> 65

<211> 212

<212> PRT

<213> Homo sapiens

<400> 65

Met Asp Tyr Leu Leu Met Ile Phe Ser Leu Leu Phe Val Ala Cys Gln
1 5 10 15

Gly Ala Pro Glu Thr Ala Val Leu Gly Ala Glu Leu Ser Ala Val Gly
20 25 30

Glu Asn Gly Gly Glu Lys Pro Thr Pro Ser Pro Pro Trp Arg Leu Arg
35 40 45

Arg Ser Lys Arg Cys Ser Cys Ser Ser Leu Met Asp Lys Glu Cys Val
50 55 60

Tyr Phe Cys His Leu Asp Ile Ile Trp Val Asn Thr Pro Glu His Val
65 70 75 80

Val Pro Tyr Gly Leu Gly Ser Pro Arg Ser Lys Arg Ala Leu Glu Asn
85 90 95

Leu Leu Pro Thr Lys Ala Thr Asp Arg Glu Asn Arg Cys Gln Cys Ala
100 105 110

Ser Gln Lys Asp Lys Lys Cys Trp Asn Phe Cys Gln Ala Gly Lys Glu
 115 120 125

Leu Arg Ala Glu Asp Ile Met Glu Lys Asp Trp Asn Asn His Lys Lys
 130 135 140

Gly Lys Asp Cys Ser Lys Leu Gly Lys Lys Cys Ile Tyr Gln Gln Leu
 145 150 155 160

Val Arg Gly Arg Lys Ile Arg Arg Ser Ser Glu Glu His Leu Arg Gln
 165 170 175

Thr Arg Ser Glu Thr Met Arg Asn Ser Val Lys Ser Ser Phe His Asp
 180 185 190

Pro Lys Leu Lys Gly Lys Pro Ser Arg Glu Arg Tyr Val Thr His Asn
 195 200 205

Arg Ala His Trp
 210

<210> 66

<211> 636

<212> DNA

<213> Homo sapiens

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<223> (1)..(639)

<400> 66

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 1 5 10 15

gga gct cca gaa aca gca gtc tta ggc gct gag ctc agc gcg gtg ggt 96
 Gly Ala Pro Glu Thr Ala Val Leu Gly Ala Glu Leu Ser Ala Val Gly
 20 25 30

gag aac ggc ggg gag aaa ccc act ccc agt cca ccc tgg cgg ctc cgc	144
Glu Asn Gly Gly Glu Lys Pro Thr Pro Ser Pro Pro Trp Arg Leu Arg	
35 40 45	
cgg tcc aag cgc tgc tcc tgc tgc tcc ctg atg gat aaa gag tgt gtc	192
Arg Ser Lys Arg Cys Ser Cys Ser Ser Leu Met Asp Lys Glu Cys Val	
50 55 60	
tac ttc tgc cac ctg gac atc att tgg gtc aac act ccc gag cac gtt	240
Tyr Phe Cys His Leu Asp Ile Ile Trp Val Asn Thr Pro Glu His Val	
65 70 75 80	
gtt ccg tat gga ctt gga agc cct agg tcc aag aga gcc ttg gag aat	288
Val Pro Tyr Gly Leu Gly Ser Pro Arg Ser Lys Arg Ala Leu Glu Asn	
85 90 95	
tta ctt ccc aca aag gca aca gac cgt gag aat aga tgc caa tgt gct	336
Leu Leu Pro Thr Lys Ala Thr Asp Arg Glu Asn Arg Cys Gln Cys Ala	
100 105 110	
agc caa aaa gac aag aag tgc tgg aat ttt tgc caa gca gga aaa gaa	384
Ser Gln Lys Asp Lys Lys Cys Trp Asn Phe Cys Gln Ala Gly Lys Glu	
115 120 125	
ctc agg gct gaa gac att atg gag aaa gac tgg aat aat cat aag aaa	432
Leu Arg Ala Glu Asp Ile Met Glu Lys Asp Trp Asn Asn His Lys Lys	
130 135 140	
gga aaa gac tgt tcc aag ctt ggg aaa aag tgt att tat cag cag tta	480
Gly Lys Asp Cys Ser Lys Leu Gly Lys Lys Cys Ile Tyr Gln Gln Leu	
145 150 155 160	
gtg aga gga aga aaa atc aga aga agt tca gag gaa cac cta aga caa	528
Val Arg Gly Arg Lys Ile Arg Arg Ser Ser Glu Glu His Leu Arg Gln	
165 170 175	

acc agg tcg gag acc atg aga aac agc gtc aaa tca tct ttt cat gat 576
 Thr Arg Ser Glu Thr Met Arg Asn Ser Val Lys Ser Ser Phe His Asp
 180 185 190

ccc aag ctg aaa ggc aag ccc tcc aga gag cgt tat gtg acc cac aac 624
 Pro Lys Leu Lys Gly Lys Pro Ser Arg Glu Arg Tyr Val Thr His Asn
 195 200 205

cga gca cat tgg 636
 Arg Ala His Trp
 210

<210> 67

<211> 143

<212> PRT

<213> Homo sapiens

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Leu Thr Ser Ala Val Ala Lys Lys Lys Asp Lys Val Lys Lys Gly Gly
 20 25 30

Pro Gly Ser Glu Cys Ala Glu Trp Ala Trp Gly Pro Cys Thr Pro Ser
 35 40 45

Ser Lys Asp Cys Gly Val Gly Phe Arg Glu Gly Thr Cys Gly Ala Gln
 50 55 60

Thr Gln Arg Ile Arg Cys Arg Val Pro Cys Asn Trp Lys Lys Glu Phe
 65 70 75 80

Gly Ala Asp Cys Lys Tyr Lys Phe Glu Asn Trp Gly Ala Cys Asp Gly
 85 90 95

Gly Thr Gly Thr Lys Val Arg Gln Gly Thr Leu Lys Lys Ala Arg Tyr
 100 105 110

Asn Ala Gln Cys Gln Glu Thr Ile Arg Val Thr Lys Pro Cys Thr Pro
 115 120 125

Lys Thr Lys Ala Lys Ala Lys Ala Lys Lys Gly Lys Gly Lys Asp
 130 135 140

<210> 68

<211> 429

<212> DNA

<213> Homo sapiens

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<223> (1)..(432)

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 Met Gln His Arg Gly Phe Leu Leu Leu Thr Leu Leu Ala Leu Leu Ala
 1 5 10 15

ctc acc tcc gcg gtc gcc aaa aag aaa gat aag gtg aag aag ggc ggc 96
 Leu Thr Ser Ala Val Ala Lys Lys Lys Asp Lys Val Lys Lys Gly Gly
 20 25 30

cgc ggg agc gag tgc gct gag tgg gcc tgg ggg ccc tgc acc ccc agc 144
 Pro Gly Ser Glu Cys Ala Glu Trp Ala Trp Gly Pro Cys Thr Pro Ser
 35 40 45

agc aag gat tgc ggc gtg ggt ttc cgc gag ggc acc tgc ggg gcc cag 192
 Ser Lys Asp Cys Gly Val Gly Phe Arg Glu Gly Thr Cys Gly Ala Gln
 50 55 60

acc cag cgc atc cgg tgc agg gtg ccc tgc aac tgg aag aag gag ttt 240
 Thr Gln Arg Ile Arg Cys Arg Val Pro Cys Asn Trp Lys Lys Glu Phe
 65 70 75 80

gga gcc gac tgc aag tac aag ttt gag aac tgg ggt gcg tgt gat ggg 288
 Gly Ala Asp Cys Lys Tyr Lys Phe Glu Asn Trp Gly Ala Cys Asp Gly

	85	90	95	
ggc aca ggc acc aaa gtc cgc caa ggc acc ctg aag aag gcg cgc tac				336
Gly Thr Gly Thr Lys Val Arg Gln Gly Thr Leu Lys Lys Ala Arg Tyr				
	100	105	110	
aat gct cag tgc cag gag acc atc cgc gtc acc aag ccc tgc acc ccc				384
Asn Ala Gln Cys Gln Glu Thr Ile Arg Val Thr Lys Pro Cys Thr Pro				
	115	120	125	
aag acc aaa gca aag gcc aaa gcc aag aaa ggg aag gga aag gac				429
Lys Thr Lys Ala Lys Ala Lys Ala Lys Lys Gly Lys Gly Lys Asp				
	130	135	140	
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20 25 30				
Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly				
35 40 45				
Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met				
50 55 60				
Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro				
65 70 75 80				
Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu				
85 90 95				

Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser		
100	105	110
Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn		
115	120	125
Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu		
130	135	140
Ser Ser Ile Pro Glu Asn Glu Ala Ile Ser Ser Ala Glu Leu Arg Leu		
145	150	155
		160
Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His		
	165	170
		175
Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro		
180	185	190
Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn		
195	200	205
Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp		
210	215	220
Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His		
225	230	235
		240
Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg		
	245	250
		255
Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu		
260	265	270
Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg		
275	280	285

Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys
 290 295 300

Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val
 305 310 315 320

Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr
 325 330 335

Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 340 345 350

Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile
 355 360 365

Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
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 385 390 395 400

Val Val Glu Gly Cys Gly Cys Arg
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<210> 70

<211> 1224

<212> DNA

<213> Homo sapiens

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<223> (1)..(1227)

<400> 70

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 1 5 10 15

ctg cta gga ggc gcg agc cat gct agt ttg ata cct gag acg ggg aag 96

Leu Leu Gly Gly Ala Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys	
20 25 30	
aaa aaa gtc gcc gag att cag ggc cac gcg gga gga cgc cgc tca ggg	144
Lys Lys Val Ala Glu Ile Gln Gly His Ala Gly Gly Arg Arg Ser Gly	
35 40 45	
cag agc cat gag ctc ctg cgg gac ttc gag gcg aca ctt ctg cag atg	192
Gln Ser His Glu Leu Leu Arg Asp Phe Glu Ala Thr Leu Leu Gln Met	
50 55 60	
ttt ggg ctg cgc cgc cgc ccg cag cct agc aag agt gcc gtc att ccg	240
Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys Ser Ala Val Ile Pro	
65 70 75 80	
gac tac atg cgg gat ctt tac cgg ctt cag tct ggg gag gag gag gaa	288
Asp Tyr Met Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu Glu Glu Glu	
85 90 95	
gag cag atc cac agc act ggt ctt gag tat cct gag cgc ccg gcc agc	336
Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala Ser	
100 105 110	
cgg gcc aac acc gtg agg agc ttc cac cac gaa gaa cat ctg gag aac	384
Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn	
115 120 125	
atc cca ggg acc agt gaa aac tct gct ttt cgt ttc ctc ttt aac ctc	432
Ile Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu	
130 135 140	
agc agc atc cct gag aac gag gcg atc tcc tct gca gag ctt cgg ctc	480
Ser Ser Ile Pro Glu Asn Glu Ala Ile Ser Ser Ala Glu Leu Arg Leu	
145 150 155 160	
ttc cgg gag cag gtg gac cag ggc cct gat tgg gaa agg ggc ttc cac	528

Phe Arg Glu Gln Val Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His	
165	170 175
cgt ata aac att tat gag gtt atg aag ccc cca gca gaa gtg gtg cct	576
Arg Ile Asn Ile Tyr Glu Val Met Lys Pro Pro Ala Glu Val Val Pro	
180	185 190
ggg cac ctc atc aca cga cta ctg gac acg aga ctg gtc cac cac aat	624
Gly His Leu Ile Thr Arg Leu Leu Asp Thr Arg Leu Val His His Asn	
195	200 205
gtg aca cgg tgg gaa act ttt gat gtg agc cct gcg gtc ctt cgc tgg	672
Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro Ala Val Leu Arg Trp	
210	215 220
acc cgg gag aag cag cca aac tat ggg cta gcc att gag gtg act cac	720
Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu Val Thr His	
225	230 235 240
ctc cat cag act cgg acc cac cag ggc cag cat gtc agg att agc cga	768
Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser Arg	
245	250 255
tcg tta cct caa ggg agt ggg aat tgg gcc cag ctc cgg ccc ctc ctg	816
Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu	
260	265 270
gtc acc ttt ggc cat gat ggc cgg ggc cat gcc ttg acc cga cgc cgg	864
Val Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg	
275	280 285
agg gcc aag cgt agc cct aag cat cac tca cag cgg gcc agg aag aag	912
Arg Ala Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys	
290	295 300
aat aag aac tgc cgg cgc cac tcg ctc tat gtg gac ttc agc gat gtg	960

Asn Lys Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val
 305 310 315 320

ggc tgg aat gac tgg att gtg gcc cca cca ggc tac cag gcc ttc tac 1008
 Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr
 325 330 335

tgc cat ggg gac tgc ccc ttt cca ctg gct gac cac ctc aac tca acc 1056
 Cys His Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr
 340 345 350

aac cat gcc att gtg cag acc ctg gtc aat tct gtc aat tcc agt atc 1104
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile
 355 360 365

ccc aaa gcc tgt tgt gtg ccc act gaa ctg agt gcc atc tcc atg ctg 1152
 Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
 370 375 380

tac ctg gat gag tat gat aag gtg gta ctg aaa aat tat cag gag atg 1200
 Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met
 385 390 395 400

gta gta gag gga tgt ggg tgc cgc 1224
 Val Val Glu Gly Cys Gly Cys Arg
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<210> 71

<211> 24

<212> DNA

<213> Artificial Sequence

<400> 71

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<210> 72

<211> 24

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<213> Artificial Sequence
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 tgcctacggt ggtgcgcct ctgc 24

<210> 73
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 <400> 73
 gaagcgcaac agggccatca cg 22

<210> 74
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 ccacgtcacg caggtcccgt tc 22

<210> 75
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 gatcctgttc tctgcctctg ga 22

<210> 76
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 <400> 76
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<210> 77
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ttcctcgtct tggccttttg g

21

<210> 78

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<212> DNA

<213> Artificial Sequence

<400> 78

gctggatctt cgtaggctcc g

21

<210> 79

<211> 19

<212> DNA

<213> Artificial Sequence

<400> 79

ggcaagctga ccctgaagt

19

<210> 80

<211> 19

<212> DNA

<213> Artificial Sequence

<400> 80

gggtgctcag gtagtggtt

19

The claims defining the invention are as follows:

1. A method for differentiating a cell into a cardiac muscle, comprising using a factor which is expressed in a cardiogenesis region of a fetus or a factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus, wherein said cell is an isolated cell which has been isolated from bone marrow or umbilical blood, and which has the potential to differentiate into at least a cardiomyocyte, wherein the cell is CD14-negative, CD29-positive, CD44-positive and CD45-negative, wherein the differentiation is accelerated by said factor.
2. The method according to claim 1, wherein the factor which is expressed in a cardiogenesis region of a fetus or the factor which acts on differentiation into a cardiomyocyte in a cardiogenesis stage of a fetus is at least one selected from the group consisting of a cytokine, an adhesion molecule, a vitamin, a transcription factor, and an extracellular matrix.
3. The method according to claim 2, wherein the cytokine is at least one selected from the group consisting of a platelet-derived growth factor, PDGF; a fibroblast growth factor-8, FGF-8; an endothelin 1, ET1; a midkine; and a bone morphogenetic factor, BMP-4.
4. The method according to claim 3, wherein the PDGF, FGF-8, ET1, midkine, and BMP-4 comprise the amino acid sequence represented by the SEQ ID NO:3 or 5, the amino acid sequence represented by SEQ ID NO:64, the amino acid sequence represented by SEQ ID NO:66, the amino acid sequence represented by SEQ ID NO:68, and the amino acid sequence represented by SEQ ID NO:70, respectively.
5. The method according to claim 2, wherein the adhesion molecule is at least one selected from the group consisting of a gelatin, a laminine, a collagen, and a fibronectin.
6. The method according to claim 2, wherein the vitamin is retinoic acid.
7. The method according to claim 2, wherein the transcription factor is at least one selected from the group consisting of Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1.
8. The method according to claim 7, wherein the Nkx2.5/Csx, GATA4, MEF-2A, MEF-2B, MEF-2C, MEF-2D, dHAND, eHAND, TEF-1, TEF-3, TEF-5, and MesP1 comprise the amino acid sequence represented by SEQ ID NO:9, the amino acid sequence represented by SEQ ID NO:11, the amino acid sequence represented by SEQ ID NO:13, the amino acid sequence represented by SEQ ID NO:15, the amino acid sequence

represented by SEQ ID NO:17, the amino acid sequence represented by SEQ ID NO:19, the amino acid sequence represented by SEQ ID NO:21, the amino acid sequence represented by SEQ ID NO:23, the amino acid sequence represented by SEQ ID NO:25, the amino acid sequence represented by SEQ ID NO:27, the amino acid sequence represented by SEQ ID NO:29, the amino acid sequence represented by SEQ ID NO:62, respectively.

9. The method according to claim 2, wherein the extracellular matrix is an extracellular matrix derived from a cardiomyocyte.

10. The method according to claim 1, wherein the cell is further CD31-negative.

11. The method according to claim 10, wherein the cell is further CD49b-negative.

12. The method according to claim 11, wherein the cell is further CD144-positive.

13. The method according to claim 12, wherein the cell is further CD49d-negative and CD102-negative.

14. The method according to claim 10, wherein the cell is further CD49d-negative and CD102-negative.

15. The method according to claim 14, wherein the cell is further CD49b-negative.

16. The method according to claim 1, wherein the cell is further CD117-positive, CD140-positive, CD34-positive, CD144-positive, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD54-negative, CD102-negative and CD106-negative.

17. The method according to claim 1, wherein the cell is further CD117-positive, CD140-positive, CD34-positive, CD144-negative, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD54-negative, CD102-negative and CD106-negative.

18. The method according to claim 1, wherein the cell is further CD117-positive, CD140-positive, CD34-negative, CD144-positive, CD90-negative, Flk-1-negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD54-negative, CD102-negative and CD106-negative.

19. The method according to claim 1, wherein the cell is further CD117-positive, CD140-positive, CD34-negative, CD144-negative, CD90-negative, Flk-1-

negative, CD31-negative, CD105-negative, CD49b-negative, CD49d-negative, CD54-negative, CD102-negative and CD106-negative.

20. The method according to any one of claims 1 to 19, wherein the cell is isolated from bone marrow.

5 21. The method according to any one of claims 1 to 20, wherein the cell is a multipotential stem cell.

22. The method according to any one of claims 1 to 21, wherein the cell is a multipotential stem cell which differentiates into at least a cardiomyocyte and a vascular endothelial cell.

10 23. The method according to any one of claims 1 to 22, wherein the cell is a multipotential stem cell which differentiates into at least a cardiomyocyte, an adipocyte, a skeletal muscle cell, an osteoblast, and a vascular endothelial cell.

24. The method according to any one of claims 1 to 23, wherein the cell is a multipotential stem cell which differentiates into at least a cardiomyocyte, an adipocyte, a skeletal muscle cell, an osteoblast, a vascular endothelial cell, a nervous cell, and a hepatic cell.

25. The method according to any one of claims 1 to 24, wherein the cell is a multipotential stem cell which differentiates into any cell in adult tissues.

26. The method according to any one of claims 1 to 25, wherein the cell is a cell which does not take up Hoechst 33342.

27. The method according to any one of claims 1 to 26, wherein said cell has the potential to differentiate into a ventricular cardiac muscle cell.

28. The method according to any one of claims 1 to 27, wherein said cell has the potential to differentiate into a sinus node cell.

25 29. The method according to any one of claims 1 to 28, wherein said cell isolated from bone marrow or umbilical blood derived from a mammal.

30. The method according to claim 29, wherein the mammal is selected from the group consisting of a mouse, a rat, a guinea pig, a hamster, a rabbit, a cat, a dog, a sheep, a swine, cattle, a goat and a human.

30 31. The method according to any one of claims 1 to 30, wherein said cell is mouse bone marrow-derived multipotential stem cell BMSC (FERM BP-7043).

32. The method according to any one of claims 1 to 31, wherein said cell has the potential to differentiate into a cardiomyocyte by demethylation of a chromosomal DNA of the cell.

33. The method according to claim 32, wherein the demethylation is carried out by at least one selected from the group consisting of demethylase, 5-azacytidine, and dimethyl sulfoxide, DMSO.

34. The method according to claim 33, wherein the demethylase comprises the
s amino acid sequence represented by SEQ ID NO:1.

Dated 21 March, 2006

Kyowa Hakko Kogyo Co., Ltd

Patent Attorneys for the Applicant/Nominated Person

SPRUSON & FERGUSON

EDITORIAL NOTE

APPLICATION NUMBER – 22281/01

Pages 147-162 are absent, the abstract follows on pages 162-163

FIG. 1

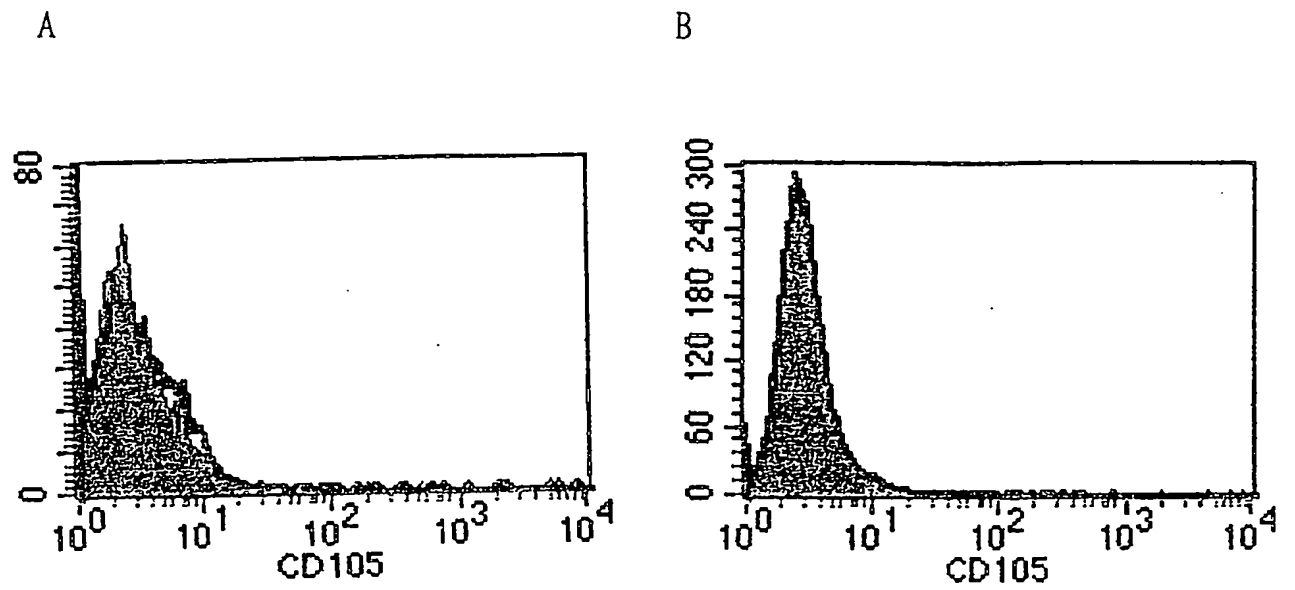


FIG. 2

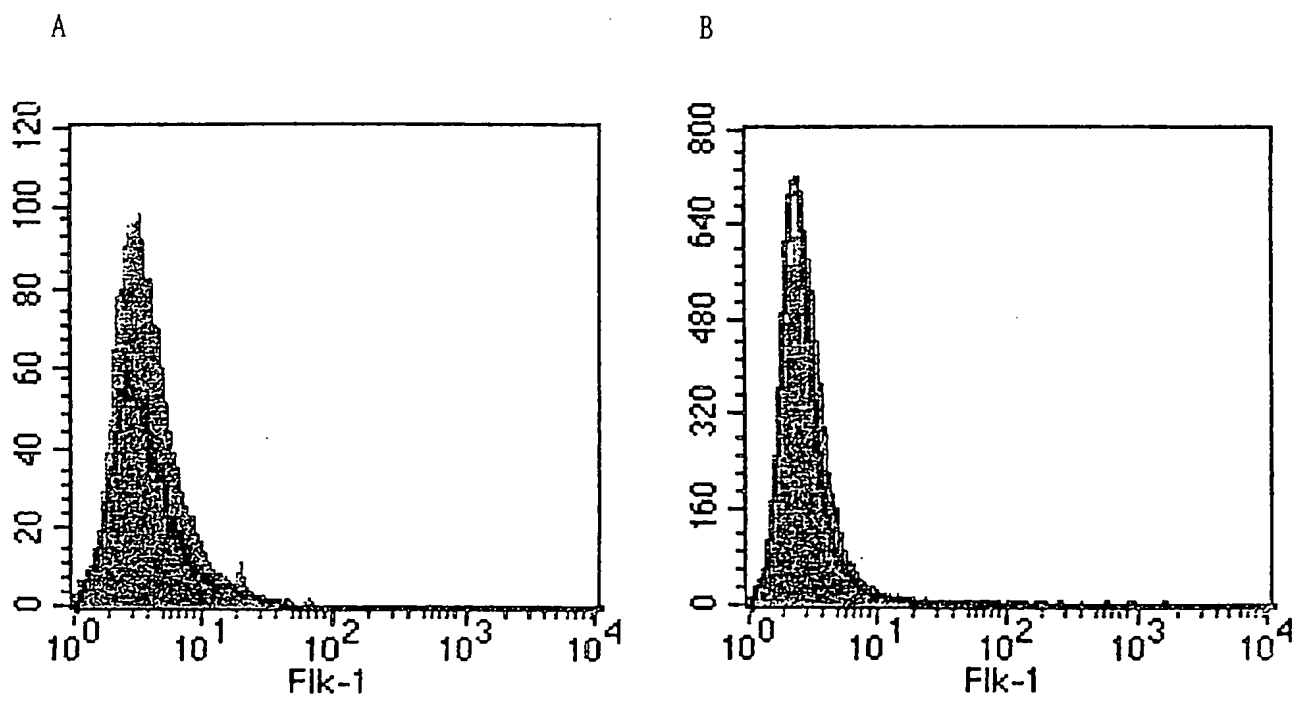


FIG. 3

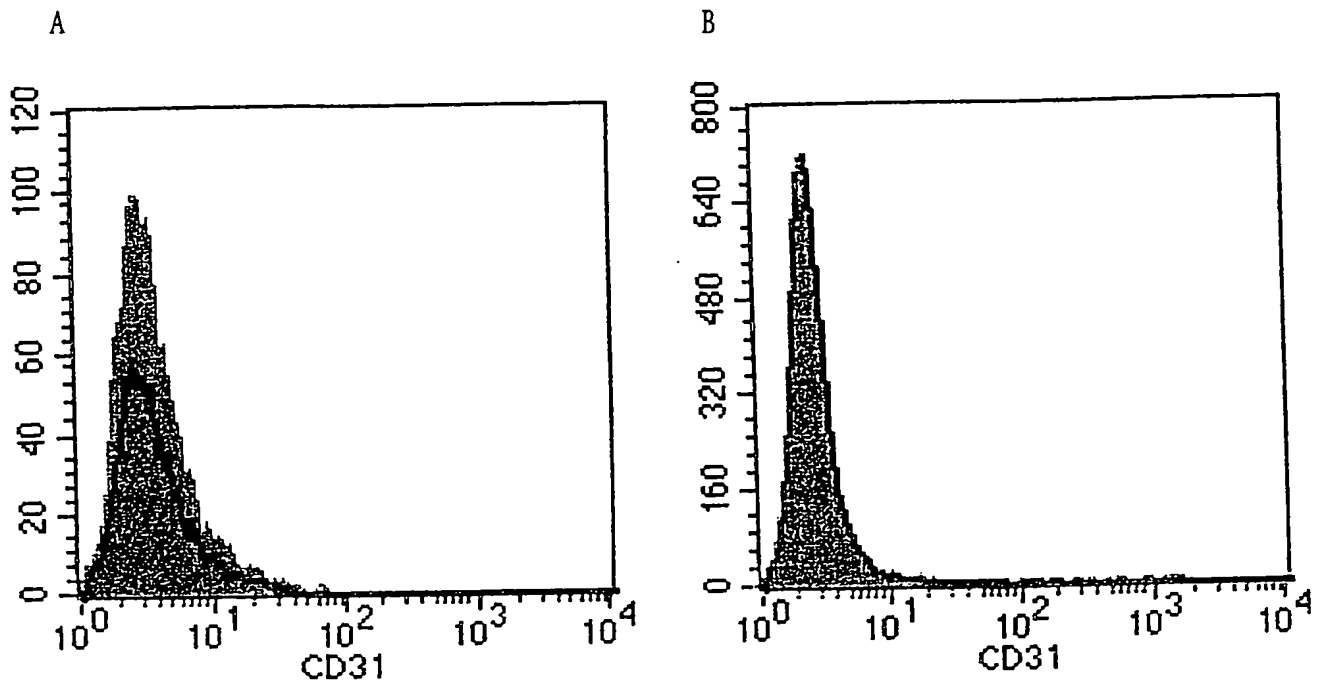


FIG. 4

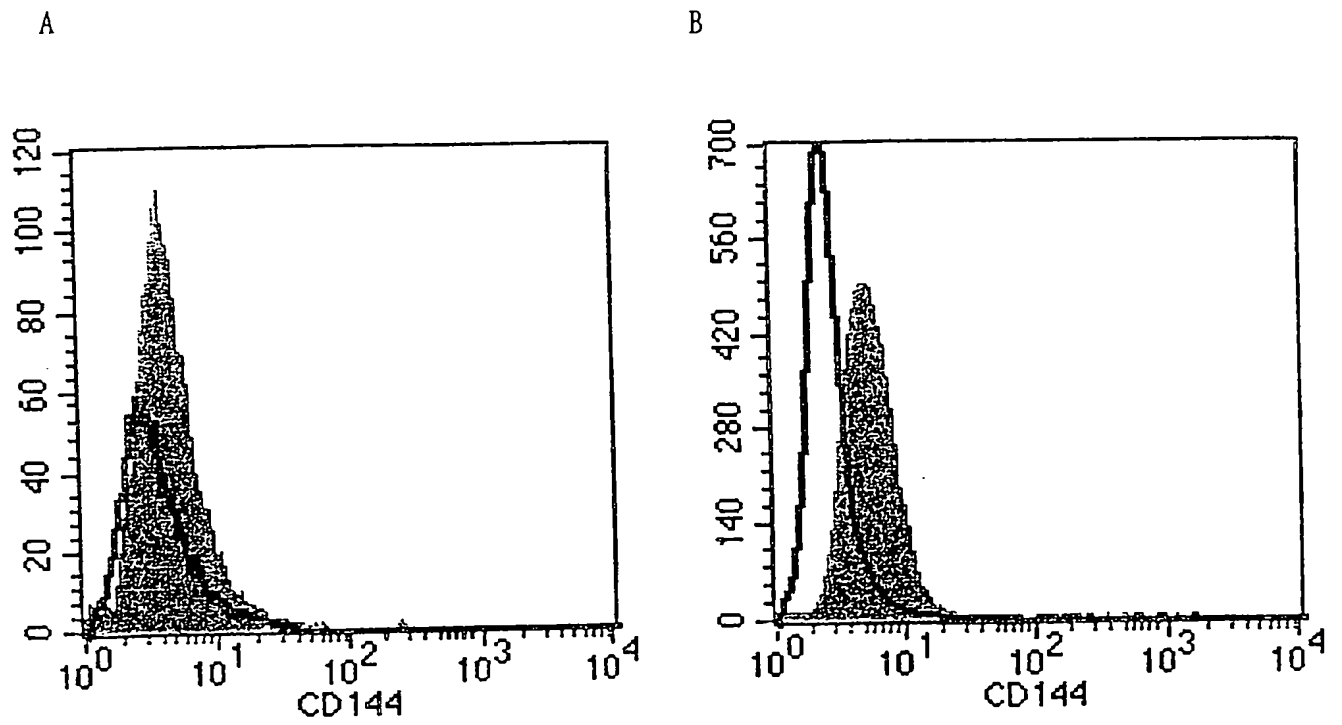


FIG 5

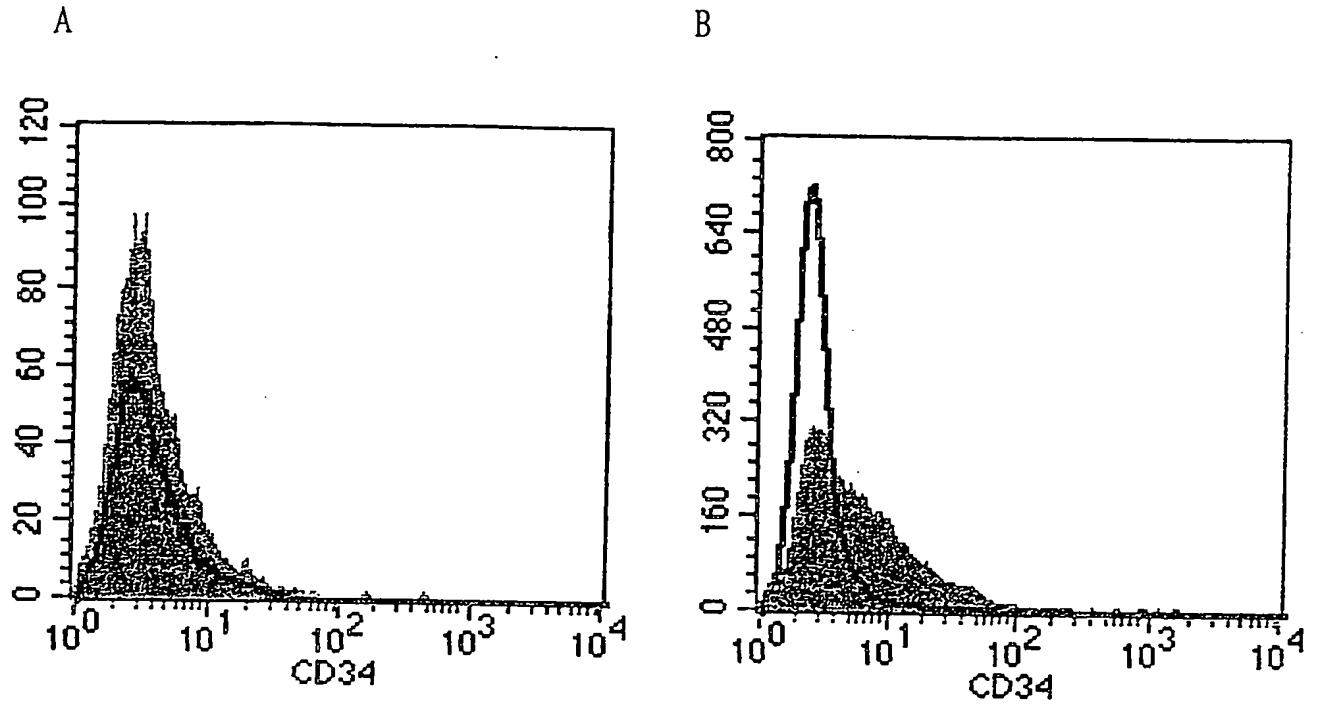


FIG 6

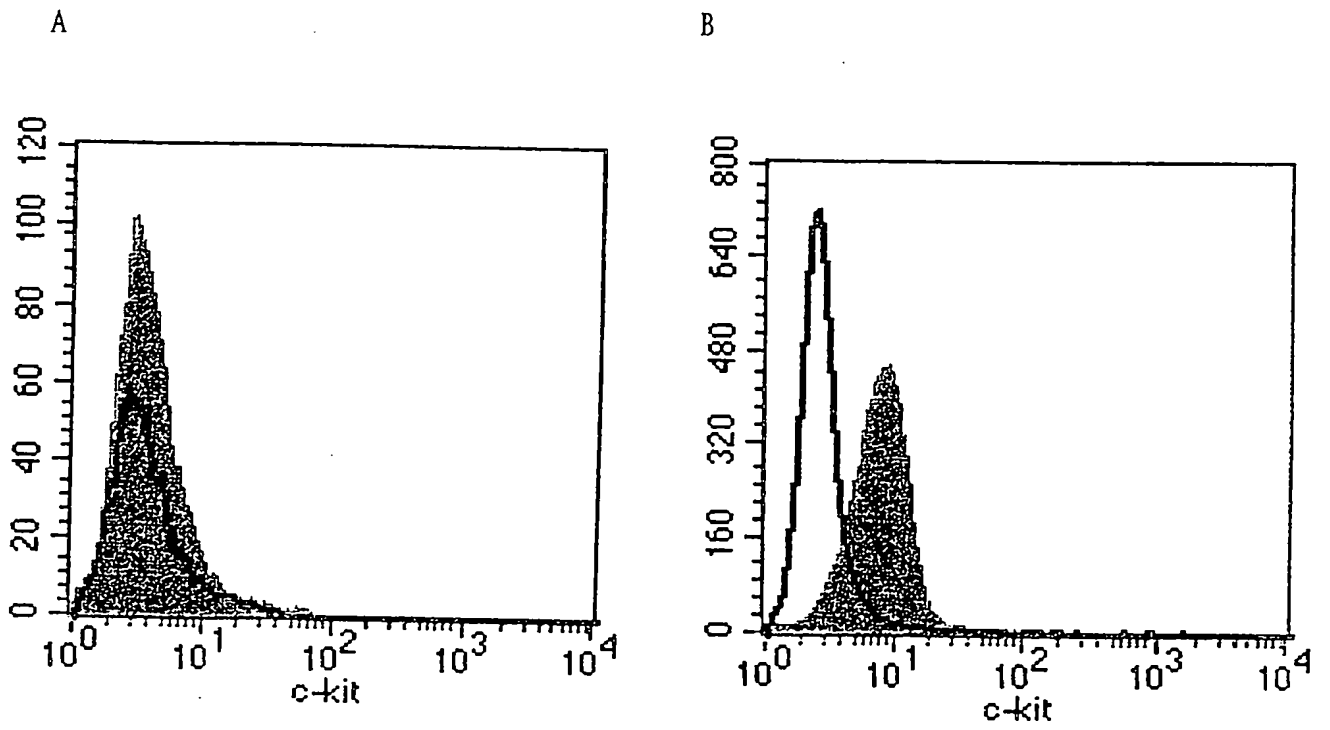


FIG 7

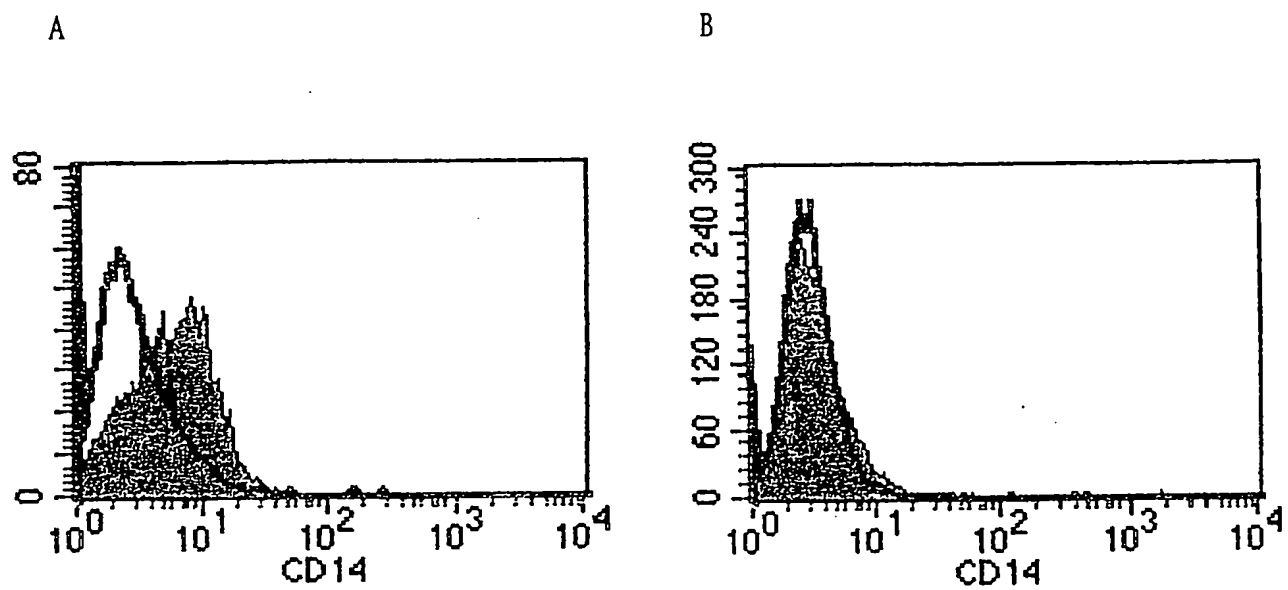


FIG 8

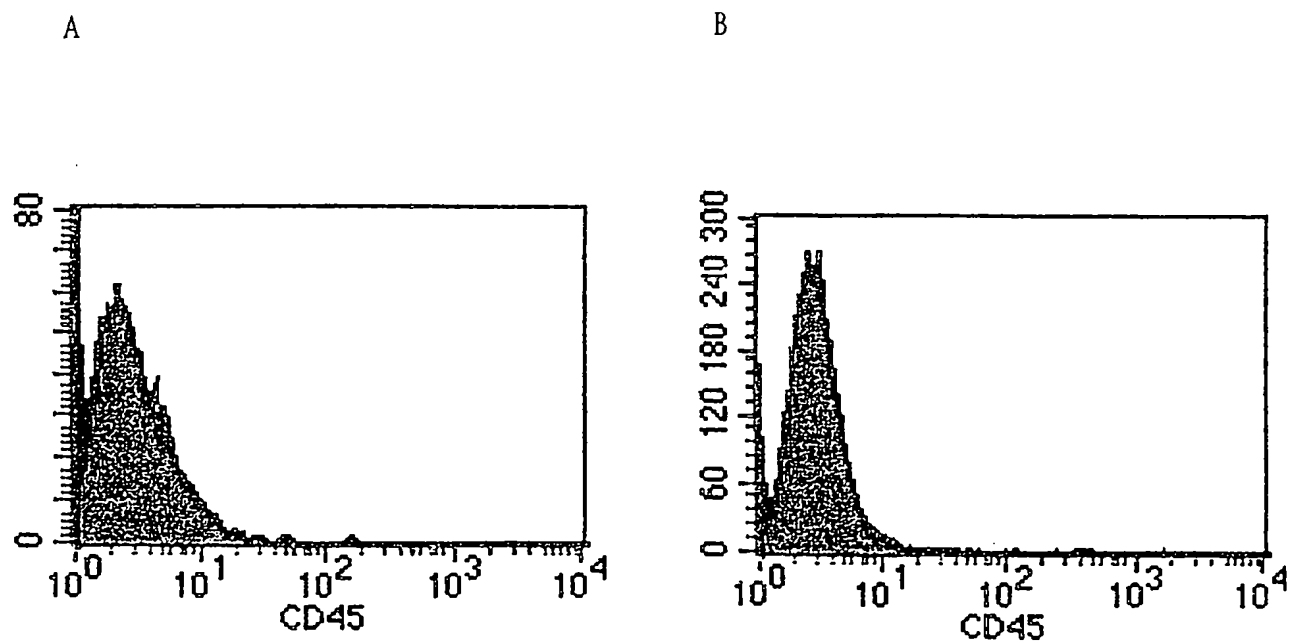


FIG 9

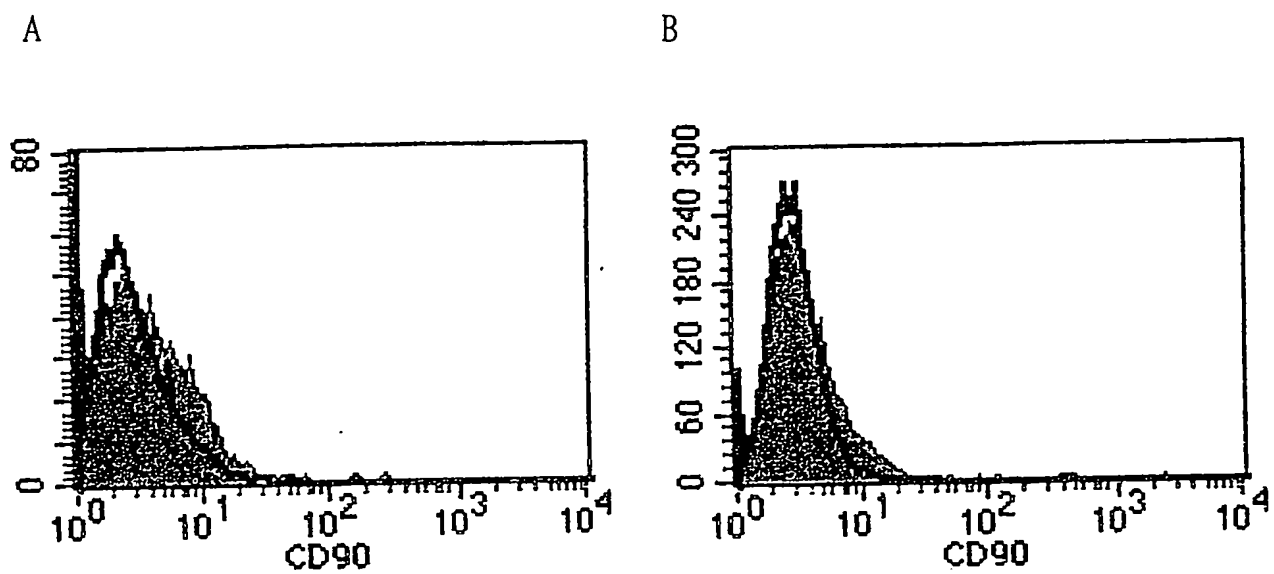


FIG 10

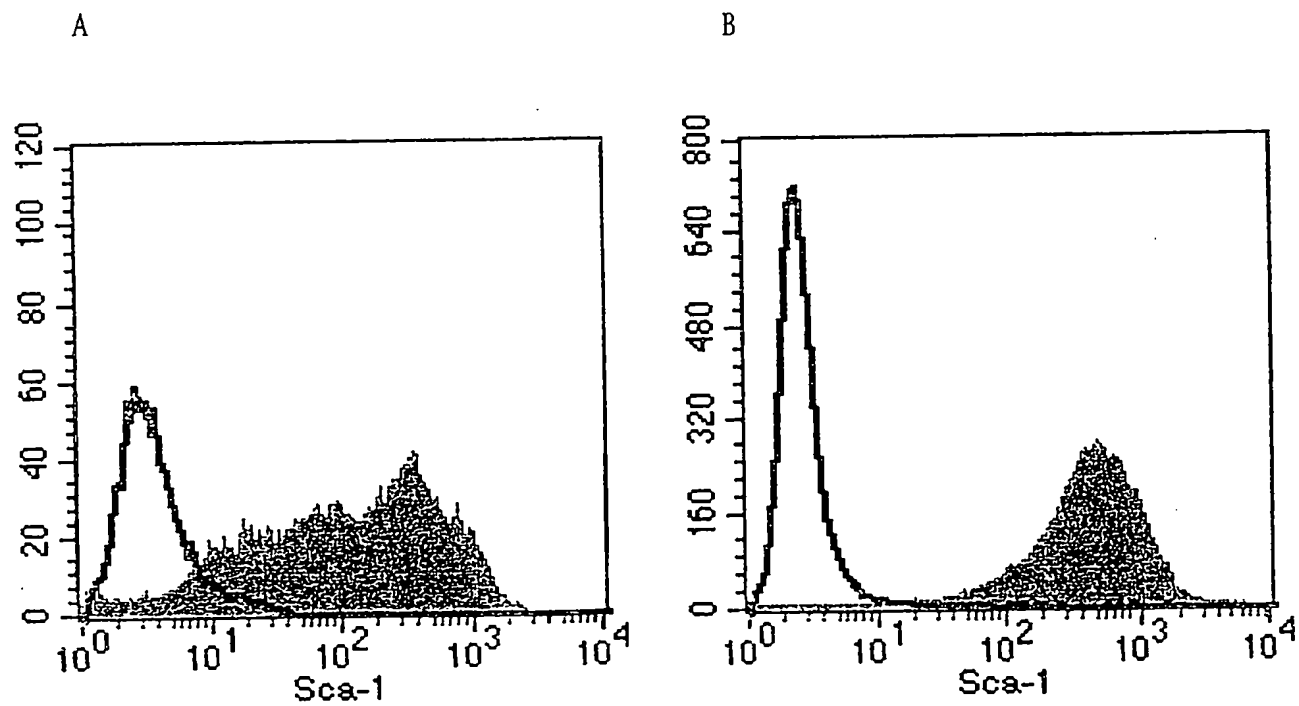


FIG 11

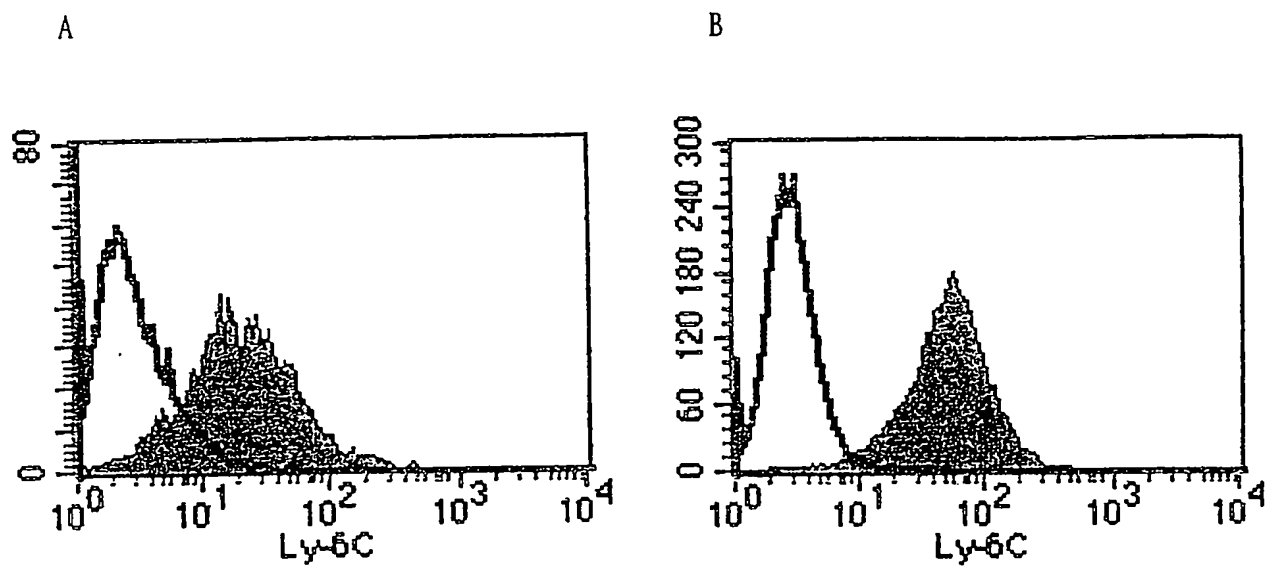


FIG 12

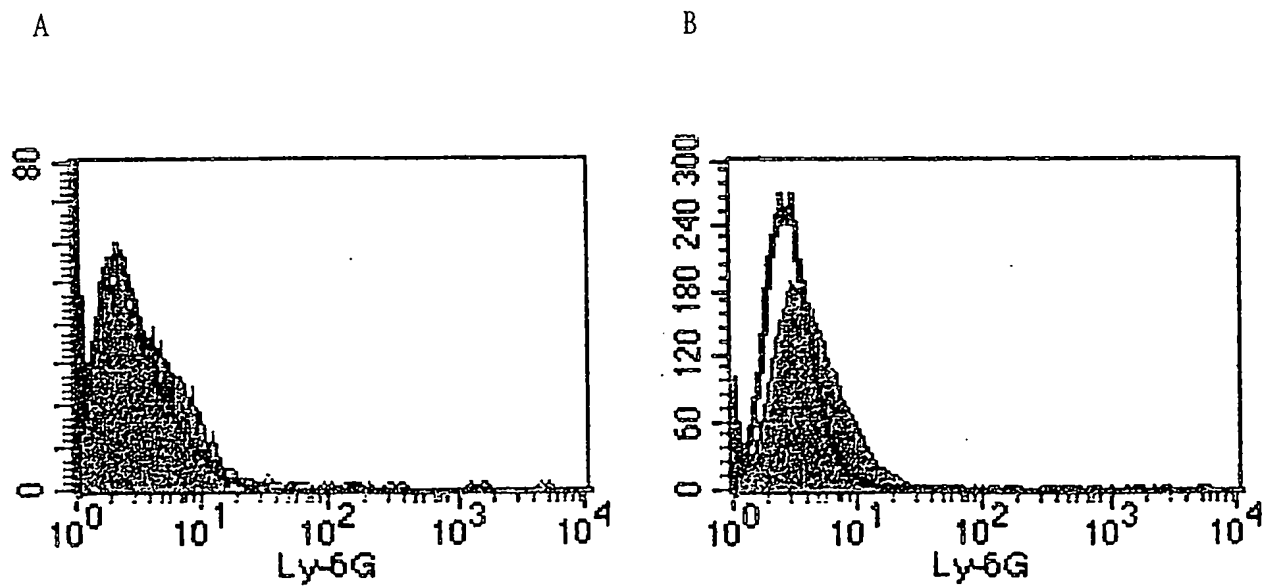


FIG 13

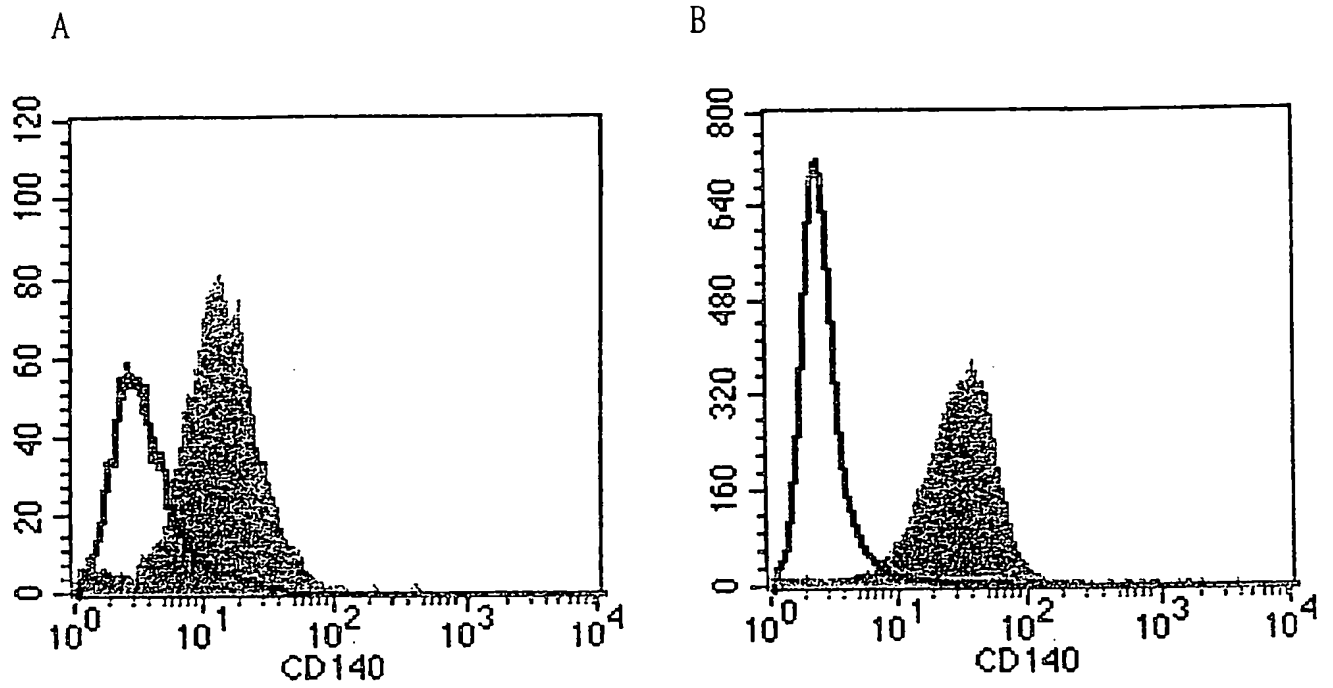


FIG 14

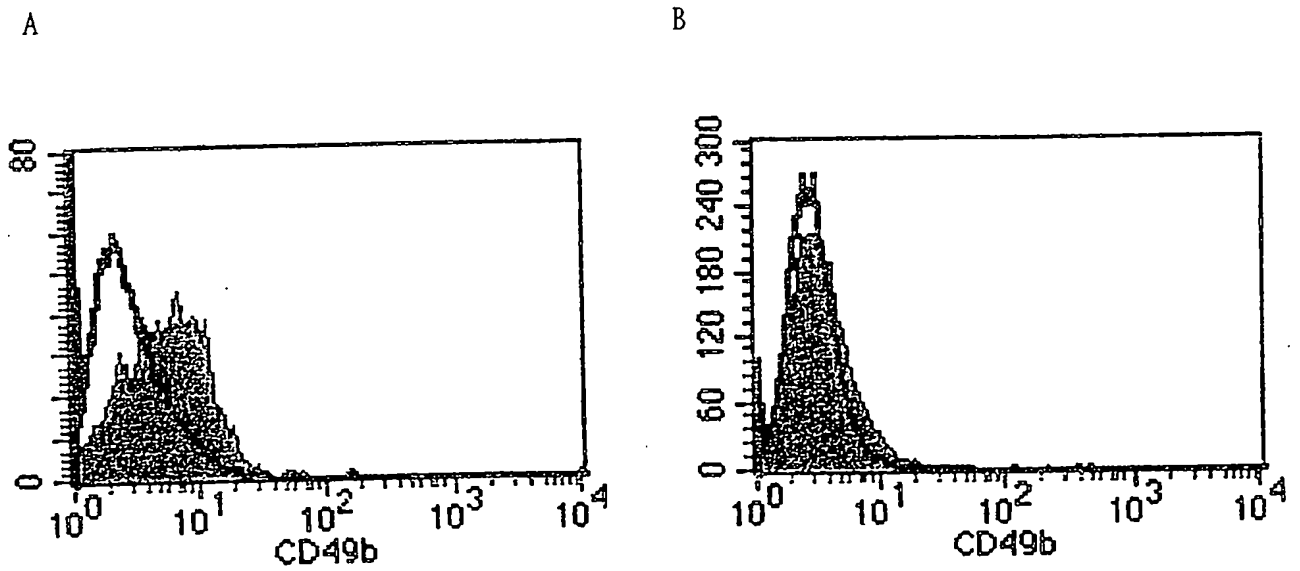


FIG 15

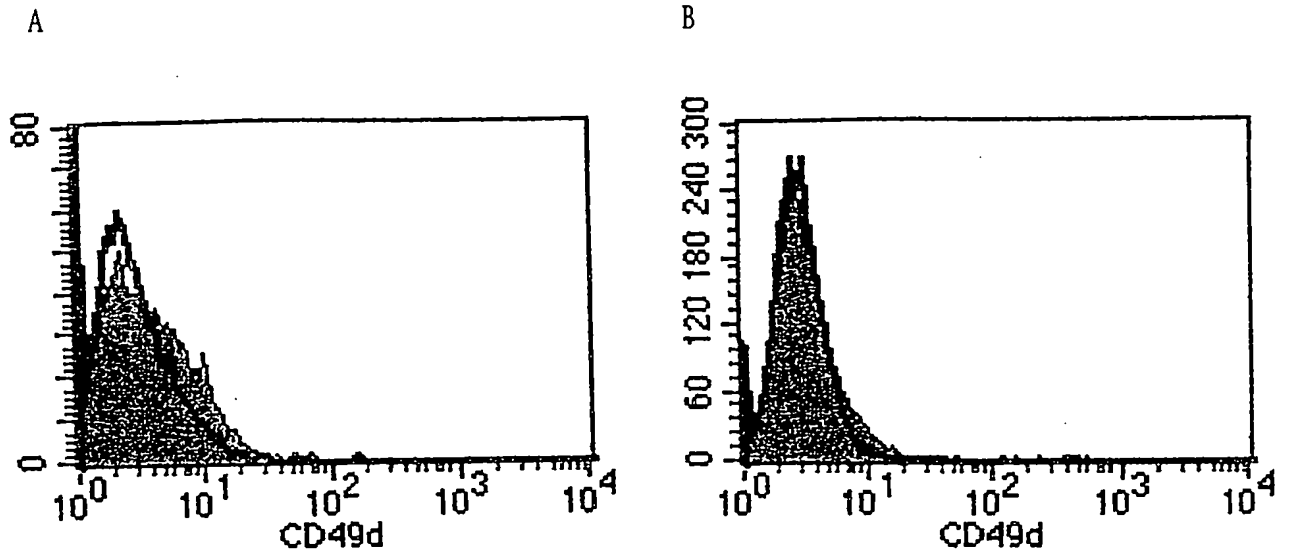


FIG 16

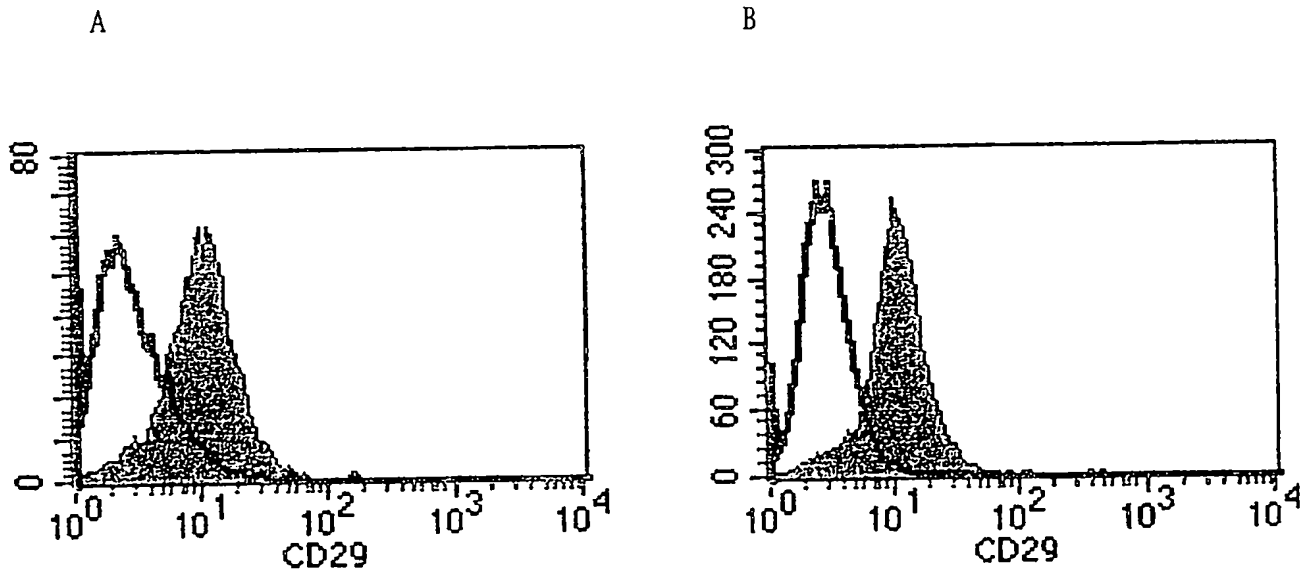


FIG. 17

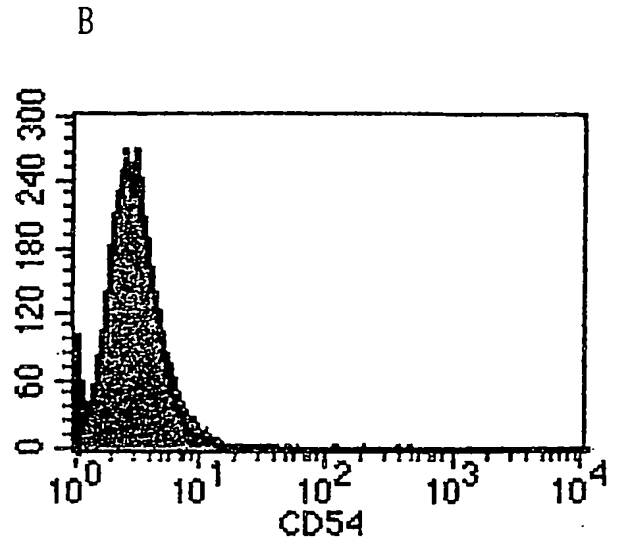
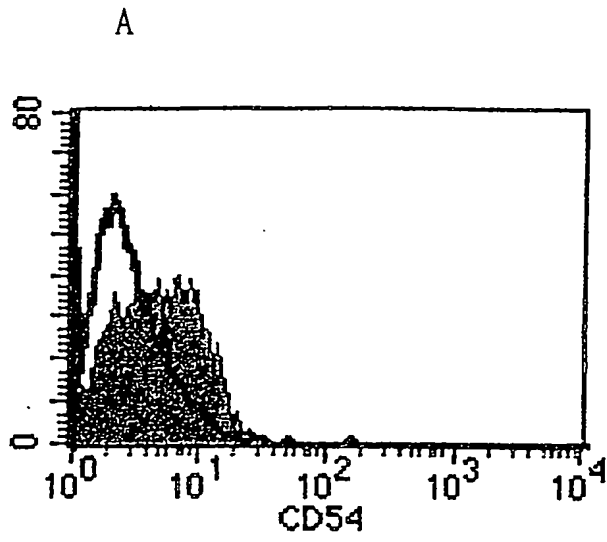


FIG. 18

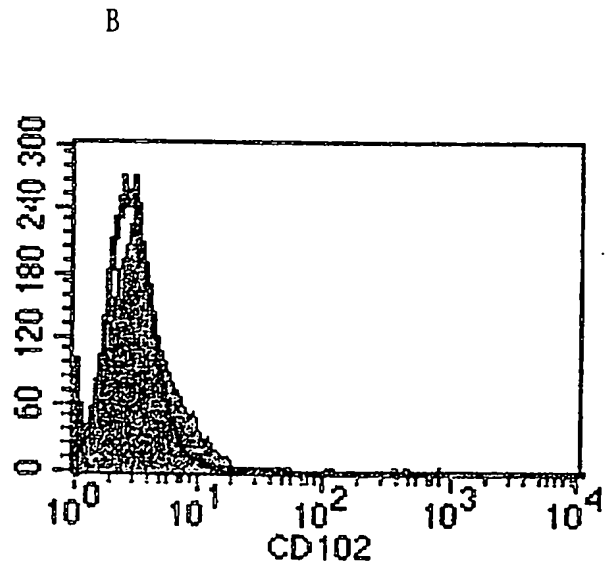
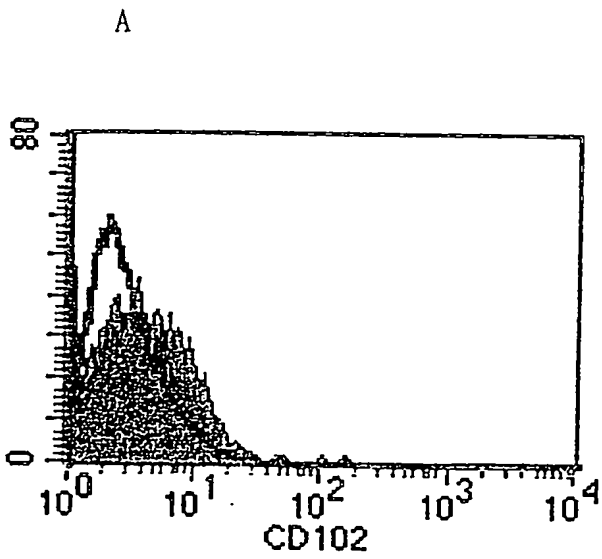


FIG. 19

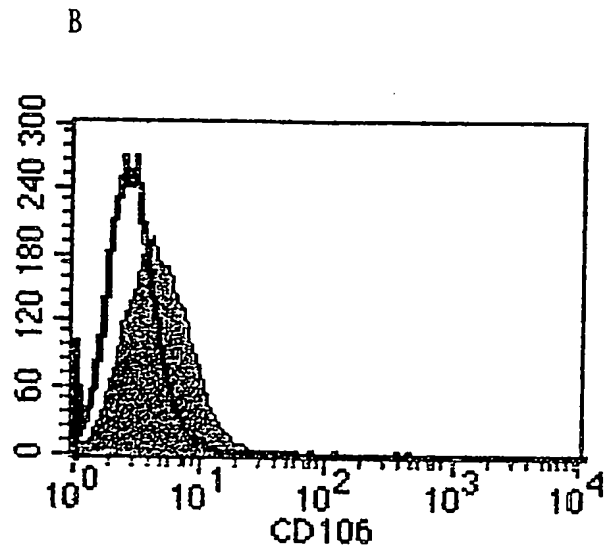
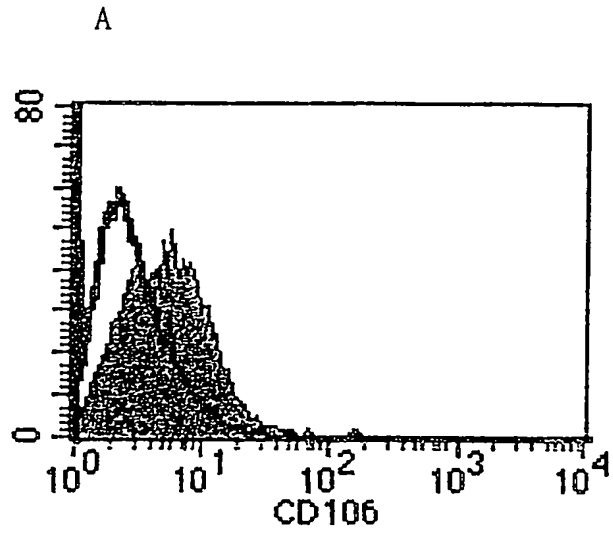


FIG. 20

